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Hæmoglobin Cells and Other Studies of the Genus *Buenoa* (Hemiptera, Notonectidæ).*

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INTRODUCTION.

THE purpose of this paper, after about one year's study, is to give what additional knowledge has been secured for the genus *Buenoa* of the family Notonectidæ. It applies especially to *Buenoa margaritacea* Bueno with its recently discovered hæmoglobin cells. While the study began with these very interesting cells, it has extended to and included life-history observations, external and internal morphological studies, genitalia, and specific characters upon which distinct species may be based.

In the *Canadian Entomologist* for November, 1922, Dr. H. B. Hungerford, of the University of Kansas, reported the presence of oxyhæmoglobin in definite cell clusters in the above-named species. This he proved by means of a spectroscopic test, the hæmin crystal test, and chemical tests such as Weber's guaiac test for blood. All the tests gave positive results, and he wrote:

* Submitted to the Department of Entomology and the faculty of the Graduate School of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Arts.

". . . And we are thus able to report that we have in this case an insect containing definite, bright red clusters of cells, enmeshed and closely associated with the tracheal system of the abdomen, which contain oxyhæmoglobin.

"In the case of *Chironomus* (the bloodworm), which has been cited frequently as the only insect possessing hæmoglobin, the red fluid is free in the body cavity, and when the larva is punctured flows out at once. Rollett (72) in 1861 discovered hæmoglobin crystals in this pigmented material and Lankester (47) in 1867 noted that it gives the characteristic absorption spectrum of hæmoglobin. Cuenot (21), 1891, who investigated the blood and lymphatic systems of many vertebrates and invertebrates, is often cited in connection with the above case."

Then follow his remarks on the only other known example besides *Buenoa* of an insect containing hæmoglobin cells in definite attached clusters, and he says:

"A case more interesting, and structurally more like the one I am reporting, is that of the larva of *Gastrophilus equi*. Berlese (3) gives a discussion of the literature relating to the discovery and study of certain pink cells related to the tracheal system in these botfly larvæ. He cites Vaney (81) as showing that the red color of these 'cellules tracheales' is due to hæmoglobin, which he appears to have thought was secured in some manner from the host. Vaney found further that the hæmoglobin tended to disappear following the larval period. Both the above-mentioned records are in the Diptera. We are pleased, therefore, to report the presence of oxyhæmoglobin in definite cell clusters in the free-swimming aquatic hemipteron *Buenoa,* and to suggest that it is, no doubt, present also in the closely allied genus, *Anisops*.* Since these insects are not parasitic, the hæmoglobin is produced by them, and has a normal physiological function to perform."

In a footnote at the bottom of the page he has stated that "a complete study of the histology and development of these interesting cells is under way." It is in accordance with this footnote that these studies have been undertaken.

Since the work started it was stated that a careful study should be made of the genitalia and other structures, for it was felt that the genus contained several other species as yet undescribed because of the dearth of specific characters upon which to base them. So, in addition, these studies have been worked out as carefully as the time and materials available have permitted, and they are grouped together in this paper with the hope that they will become a valuable part of the knowledge of insect life.

Much appreciation is due to Dr. H. B. Hungerford for the materials he has loaned, for his kindly suggestions and encouragement,

* See Poisson: *Anisops producta* Fieb., in Archives de Zoölogie Experimentale, T. 65, Fas. 4, pp. 182-208; 1926. Since the completion of this thesis, ten pages and six figures are devoted by this European worker to the hæmoglobin cells or "cellules tracheales" of the genus *Anisops*.

and for his guidance in the conduct of these studies; also, to Dr. P. B. Lawson for his aid in the morphological studies; and to Miss Kathleen Doering, Mr. P. A. Readio, Mr. R. H. Beamer, Mr. William Robinson, or any others who may have assisted in any way or been interested in these problems.

In order to more fully understand the group of insects here considered, it may be well to mention the more important fact of their phylogenetic and systematic relationship, life history, and other points of more general interest before beginning with the more specific parts of the investigation. The Notonectidæ belong to that great group of the Hemiptera-Heteroptera, sometimes called the Cryptocerata, and seem to be closely related to the Corixidæ.

TAXONOMY.

In University of Kansas Science Bulletin, volume XI, page 165, Doctor Hungerford discusses the family Notonectidæ as follows:

"The family Notonectidæ embraces, according to Kirkaldy, two subfamilies, Pleinæ and Notonectinæ. In his 'Revision of the Notonectidæ, part I,' in Trans. Ent. Soc. London (3), Vol. 35, pp. 393-426, 1897, this author begins his systematic revision of the family. This is continued in a second paper which appeared in the 'Weiner Entomologischen Zeitung' for 1904, and entitled 'Uber Notonectiden.'

"In the following year Bueno published 'The Genus *Notonecta* in America North of Mexico.'

"Kirkaldy lists six genera in the subfamily Notonectinæ: *Notonecta* L., *Anisops* Spin., *Enithares* Spin., *Martarega* B. White, and *Nychia* Stal. It is in his second paper that he erects the genus *Buenoa* which is allied to *Anisops*. In this country we have the three genera: *Notonecta, Buenoa* and *Plea*. These may be separated by the following:

KEY TO GENERA.

A. Legs quite similar ..... *Plea* Leach.
AA. Legs dissimilar, hind legs flattened and fringed for swimming.
  B. Last segment of antennæ much shorter than the penultimate..... *Notonecta* Linn.
  BB. Last segment of antennæ longer than the penultimate..... *Buenoa* Kirk.

"Of these three genera *Notonecta* is the commonest, being represented in this country by 12 species; the genus *Buenoa* comes next, with 5," (now 7), "while the little *Plea* so far is credited in the literature with but 1."

Since the above was published Doctor Hungerford has described two other species, and the genus *Buenoa* now contains 7 species instead of 5 in this country.

Dr. Carl Drake has since described *Plea harnedi:*

In the same publication on page 173 the genus *Buenoa* is described as follows:

"Genus *Buenoa* Kirkaldy.

"Named in honor of Mr. J. T. de la Torre Bueno.

"Similar to the genus *Anisops,* but the male is provided with two tarsal segments on the front legs, and claws are differently formed.

"Head: Eyes not contiguous, but interior margins about parallel, their margins curving slightly, making distance at vertex greatest, and interocular space long and narrow. Labrum reaching to apex of second rostral segment; last segment of antenna longer than the penultimate.

"Thorax: Pronotum not most transverse; alæ present and the hemelytra divided into clavus, corium and membrane, and more transparent and hyaline than in *Notonecta.* Hind femora not reaching apex of hemelytra. The hind tarsi are provided with more conspicuous claws than *Notonecta.* The middle and front legs are armed with long spines and the males of *Buenoa elegans* and *Buenoa margaritacea* at least are equipped on the front leg with a tibial structure borne on the inner face of a prominence, which is forced into a thin, elongate spur by the elevation of the inner angle or margin of the tibia near its base. Tarsi of front legs two-segmented in both sexes.

"Abdomen: The abdomen is provided with a median ventral carina and the lateral margins of the venter are provided with guard hairs which cover the 'gutters.' The female has the last ventral abdominal segments modified for sheathing the ovipositor, which consists in the main of a pair of somewhat spatulate chitinized gonapophyses for placing the eggs in the tissues of plants."

The following key is proposed to include the species now found and described in the United States. It is probable that *B. platycnemis* (Fieber) does not exist here.

KEY TO *BUENOA.*

A. Scutellum broad and as long or longer than the pronotum.
  B. Pronotum in the male with four depressions, appearing tricarinate.
    C. Head with eyes in male nearly as wide as pronotum, a little narrower in female; legs robust; length 7.5 to 9 mm. ..... *B. carinata* (Champion)
    CC. Head with eyes somewhat flattened and distinctly narrower than pronotum in both sexes; legs slender; pale color; length 6.5 mm. *B. albida* (Champion)
  BB. Pronotum almost unimpressed, lateral carinæ in some males.
    C. Small oval stridular area on front femur of males; length 6.7 to 8.1 mm. ..... *B. margaritacea* Bueno
    CC. Long sword-shaped stridular area across front femur of males; length 6.5 to 7.5 mm. ..... *B. scimitra* n. sp.
AA. Scutellum reduced and narrow and not as long as pronotum.
  B. Species over 6 mm. long.
    C. Pronotum of male large, inflated, and smooth; length 6.25 to 7 mm. *B. limnocastoris* Hungerford
    CC. Pronotum of male tricarinate; large tibiæ in front; length well over 6 mm. ..... *B. macrotibialis* Hungerford
  BB. Species less than 6 mm. long ..... *B. elegans* (Fieber)

BIOLOGY OF THE GENUS.*

Concerning the biology and life history of the species of the genus *Buenoa* apparently very little has been written except about *Buenoa margaritacea* Bueno. All the notes found are those by Doctor Hungerford (36 and 37). The observations added herein are confined to the above or very nearly related species.

EGGS AND OVIPOSITION.

Concerning the eggs the statement is confirmed that they are placed in the tissues of plants with a bit of the surface exposed. Most of those collected have been from a species of *Ceratophyllum* growing in a permanent pond underneath a Union Pacific railroad bridge about two miles north of Lawrence, Kan. At times only a single egg here and there has been found in the stem of this plant, nearly always with the cephalic end of the egg toward the bottom of the plant. The portion of the stem chosen varied from within a few inches of the tip of the growing stem to near the base of the plant where the stem was tougher, larger, and much discolored. Usually the eggs were more numerous, and most frequently found near the middle of the growing stem where the tissue was neither too hard nor too soft, and apparently of just the right consistency for oviposition.

Of the hexagonal reticulations of the egg surface mentioned in University of Kansas Science Bulletin, volume XI, page 195, it is found for the eggs of *B. margaritacea* that these reticulations are confined to the area immediately surrounding the micropyle or area exposed as the egg lies *in situ*. Each hexagonal area has a finely corrugated or granular appearance. The rest of the chorion appears quite smooth.

Specimens of *B. margaritacea* collected from Smith's pond one mile east of Lawrence on April 15, 1924, were placed in an aquarium jar with a long stem of *Ceratophyllum* and left sitting in a window of the laboratory. Two eggs were first observed deposited in the stem on the morning of April 24. On April 28 they were observed under the binocular, and it was found that the red eyespots were well developed. On the morning of April 30 they were found to have hatched and died almost immediately for some unknown reason.

After that date the eggs were found in the pond, and stems were brought to the laboratory and examined to find that the eggs had

* See, also, Bare: Life Histories of Some Kansas Backswimmers, pp. 93-101, Annals Ent. Soc. Am., Vol. XIX; 1926.

already hatched as early as May 5. Around May 10 eggs were quite easily found in the pond or secured by bringing live bugs from the ponds and placing them in an aquarium jar together with some *Ceratophyllum*. Most of the eggs were laid the first day or two after the bugs were brought into the laboratory. Then the bugs died one by one, until within three weeks they were all dead. The cause of their death was not determined. It may have been lack of proper food, the growth of a scum over the surface of the water in the aquarium jars, the fact that they had laid their eggs and were dying normally, or other reason. They do not appear to die in this manner in the ponds.

In the spring of 1923 the eggs were found from May 12 to June 6. No doubt they were laid much earlier than the first findings. Search was made for them last on about July 1, but none were found. But at that time many nymphs of all stages were found, and during the early days of September and as late as October 22 fifth-instar nymphs were collected from ponds about Lawrence. It seems probable, then, that egg laying continues from as early as April 24, to sometime in August, and there may be more than one generation in a season.

The egg stage from time of oviposition to hatching is about one week. At this writing only one hatching has been secured from eggs the date of oviposition of which was nearly known. From eggs collected in May, 1923, those which hatched would seem to verify the above statement.

Observations on the oviposition of *B. margaritacea* are as follows: At 7:20 a. m. May 15, 1924, a bright, sunny, cool morning, in an aquarium jar sitting in the shade at an east window through which the sun was shining, a female was first observed depositing her eggs in the stem of a species of *Ceratophyllum*. The stem was inclined at an angle of about 45 degrees at the place where the egg was deposited. The bug when first observed was standing above the stem back upward, so that the body and stem formed an angle of about 30 degrees. Other observations later showed the bug on nearly every side of the stem, and the position of the stem seemed to make little difference. Sometimes two eggs were laid almost side by side or just opposite each other in the same stem. The front pair of legs were fully extended almost perpendicular to the line of the body and grasped the stem of the plant rigidly. The middle, or mesothoracic, legs were extended caudally and grasped the stem at an angle of about 45 degrees with the line of the body. They were not fully

extended, but stood in an elbowlike position allowing considerable freedom of motion. The hind, or metathoracic, swimming legs extended laterally like balancing apparatus and ready to make the swimming stroke as normally. The tip of the abdomen was extruded and bent downward to the stem. The last exterior or seventh abdominal sternite, which has a sharp pointed caudal tip, was standing at an angle of about 40 degrees to the line of the body and was inserted into the plant tissues like a wedge.

The two sicklelike blades of the ovipositor were digging simultaneously into the stem with a backward scooping motion, and under the binocular the fragments of plant tissue like sawdust were seen floating away in the water. The movement of the ovipositors seemed to be due partly to a slight backward and forward movement of the entire body and partly to the movements of the muscles of the eighth and ninth segments of the abdomen. As the work progressed the ovipositors were inserted deeper and deeper, hollowing out the space downward and backward for the insertion of the egg, and the seventh sternite, acting as a wedge, was shoved deeper into the stem. When the opening was ready the two black-tipped styli at the tip of the abdomen were pushed against the tissue on either side of the more caudal part of the incision to help keep it open. The ovipositors were apparently used literally as levers against the edges. The bug rested for a moment, and then the egg was pushed into its receptacle by contraction of the opening of the oviduct a little above and between the ovipositors. The bug rested a few seconds longer, and then withdrew the ovipositors and sternite and swam away.

The aquarium jar in which the above observations were made contained about 40 or 50 bugs taken the evening before and placed in the jar about 4:30 p. m., together with a stem of *Ceratophyllum* about 15 inches long collected several weeks before, and in which no eggs had been previously deposited. At 7 p. m. of the same evening it was observed that the stem contained 3 eggs freshly deposited. At 7 a. m. of the next morning it contained at least 18 eggs. During the morning and again in the afternoon other bugs were seen depositing eggs. The day was partly cloudy and at about 4:30 p. m. it became quite cloudy. After that no more eggs were laid. The weather conditions seem very much to affect their activities in this respect as well as in taking flight, which is described on another page. Just previous to the clouding over at 4:30 p. m., between the hours of 2:30 and 4:30 p. m., egg laying was quite active, and most of the members of the department had an opportunity to observe it.

The time of oviposition was taken repeatedly and found to be about one and one-half minutes. Sometimes the bug would leave the place where she had been working with her ovipositor. Sometimes she seemed to be disturbed by her observers (all the *Buenoa* are quite wary) and again it seemed that the place sought did not prove satisfactory. After a trial was made of a place she would always go to the surface for a fresh supply of air before trying another place. Then she would swim quietly around till a suitable place on the stem between two nodes was found. Next she would quickly turn and grab it with the two pairs of front legs, and begin to test it with the tip of the seventh sternite and the ovipositors. If it was found suitable she would not change her position, but if not she might crawl along the stem for some distance until she found a better place. The ovipositors began working immediately when she took hold of the stem. The time required to make the opening complete for the egg was almost precisely one minute. Then she would rest about five or ten seconds and lay the egg. After another rest of ten to twenty-five seconds she would swim away. Fifteen separate observations were made of the process of oviposition in one day, and it has been noted repeatedly since.

Sometimes the bug encountered difficulty with the air contained in the cells or vessels of the plant tissue. When she was through laying the egg and started to swim away she found that a large air bubble had collected, apparently from the pierced plant cells, under the tips of her hemelytra. Then she would swim about for a time somewhat like a dog with a tin can attached to its tail until the bubble was absorbed in the water or lost at the surface. It is not certain whether the bubble of air came from the plant tissue, but it appeared to do so. It may have come from beneath the hemelytra.

It is not yet determined whether the same bug lays more than one egg in a day. From dissection it is known that the abdomen of the female sometimes contains from ten to fifteen eggs of nearly the same stage of development. It is not known how many eggs a single female may lay.

NYMPHS.

Concerning the nymphs little is added to that already known and written. Attempt is being made to further trace out the life history and length of the instars. If the success is no better than former experiences it will probably get them through to the second instar, and then all will die from confinement, improper food, or other such cause.

IDENTIFICATION.

For the purposes of aiding in identification of the different instars and distinguishing the sexes of both adults and nymphs, the following notes are given and the reader referred to pages 195-197 of the University of Kansas Science Bulletin, volume XI, for a table containing other measurements and information. The notes on the following pages apply, except as to size, to *Buenoa margaritacea*, our most common species. They may apply in nearly all other characteristics to any of the *Buenoa* species. In all cases of nymphs and adults the seventh abdominal segment appears superficially to be the sixth abdominal segment instead, because the first two abdominal segments next the thorax are so fused together that it takes careful study to distinguish them.

ADULTS.

MALES:

1. V-shaped base of beak. (On the outer faces of this V-shaped segment are sound devices.)
2. Sound devices on front tibia, femur and coxa.
3. Black glandular spot near the junction of the pleurites of the first and second abdominal segments.
4. Black spine on sinistro-caudal margin of the seventh tergite.
5. Asymmetrical genital claspers, left or sinistral one larger and hooked at the end.
6. Seventh abdominal sternite somewhat rectangular.
7. Eighth abdominal sternite visible ventrally.

FEMALES:

1. Seventh abdominal sternite projects caudad into a V-shaped tip beneath the ovipositors and tip of the abdomen.
2. Symmetrical, sword- or sickle-shaped, saw-toothed ovipositors.
3. Seventh abdominal tergite symmetrical.
4. Eighth abdominal sternite not visible ventrally.
5. Styli visible near tip of abdomen, and black tipped.

NYMPHS.

A distinguishing feature of all the instars of the nymphs to separate them from the instars of *Notonecta* nymphs with which they are likely to be confused, is that the abdominal spiracles appear large and of the same general appearance as in the adult *Buenoa*. Those of *Notonecta* are small and inconspicuous.

There are also other structural differences which are noticeable. After one has studied the ventral plates of the seventh abdominal segments he can usually be pretty sure of the sex and instar to which a nymph belongs. This is especially true after the second instar. For appearance of the ventral plates of the fifth-instar nymphs

see plate LVII. The females are usually larger and wider between the eyes than the males. This is also true of the adults. In all instars the beak is four-segmented, and tarsi are one-segmented and have two prominent claws. The tarsi of the adults are all two-segmented and the tarsal claws of the swimming legs are inconspicuous.

FIFTH-INSTAR NYMPH:

1. About 5.175 mm. long, etc.
2. Wing pads extend full length of the metathorax or to the distal ends of the hind coxæ.

Males: Seventh abdominal sternite somewhat rectangular.

Females: Seventh abdominal sternite triangular at tip.

FOURTH-INSTAR NYMPH:

1. About 4.5 mm. long, etc.
2. Wing pads extend to middle of metathorax or to distal end of mesothoracic tibiæ when flexed.

Males: Seventh abdominal sternite rectangular.

Females: Seventh abdominal sternite triangular.

THIRD-INSTAR NYMPH:

1. About 3.225 mm. long, etc.
2. Wing pads mere buds, or extending to line or trochanter of fore leg flexed.

Sexes rather hard to distinguish but some difference similar to the fourth and fifth instars.

SECOND-INSTAR NYMPH:

1. About 2.25 mm. long, etc.
2. A kind of inwardly projecting pleural fold along sides of thorax and abdomen.

Sexes indistinguishable externally.

FIRST-INSTAR NYMPH:

1. About 1.85 mm. long, etc.
2. Abdominal fringe of hairs forming ventral air chamber conspicuously extended forward along sides of thorax to the head.

Sexes indistinguishable externally.

ADULTS.

(See, also, page 273.)

For a discussion of the biology of the adults, see pages 177, 194, 195, 197 and 198, of volume XI of the University of Kansas Science Bulletin. There is hardly a living creature of the waters which appears more beautiful and graceful than does *B. margaritacea* Bueno as it calmly glides about in an aquarium jar among branches of *Ceratophyllum*. The pearly luster of the wing covers as seen with the aquarium in a window is beautifully enhanced by the film of air contained underneath them, and the perfect poise of the animal is an expression of grace worthy of note.

In the above-named reference, page 197, it is stated that "the adults appear from early spring to late fall." They are found to appear during every month of the year if one seeks them in their proper habitat. They overwinter as adults only, lay their eggs in the spring and summer, and it may be there are two generations in a season in Kansas. At intervals of every ten days or two weeks they have been collected all winter during the season of 1923-'24. The winter has been a rather unusually cold one.

On January 22 the ice, which was almost twelve inches thick on the pond at the Union Pacific railroad bridge mentioned on page 269, was cut and a few sweeps of the water net made beneath the ice and some distance from the bottom of the pond. Three specimens of *B. margaritacea*, a number of small corixids, and one *Notonecta undulata* were taken. The specimens immediately were brought to the laboratory and placed in an aquarium jar in tap water, which must have been several degrees warmer than that in the pond. The water of the pond must have been at 4 degrees Centigrade. The behavior upon this change of habitat was a little unusual. The bugs were not further chilled as they were brought into the laboratory, because it was quite a warm, pleasant day with a temperature of near 45 degrees Fahrenheit. The *Buenoa* acted stunned and very languid, and did not come to the surface for air for about one-half hour. This experience was later duplicated.

The question of just how respiration can take place under the ice and at such temperatures is an interesting one. The bugs most certainly can not get air from the surface. It is true that at 4 degrees Centigrade, the water contains its maximum of oxygen and other gases. But it hardly would seem there could be a sufficient amount for the needs of respiration, although the movements of the water creatures and bugs at such a low temperature is slight and rather languid. In this pond at the time there were some green algæ and *Ceratophyllum,* which no doubt produced some free oxygen bubbles and the water bugs may have used them. But later in February not even green plants were present. Wesenberg-Lund (88) has published a paper, "Uber die Respirations-verhaltnisse bei unter dem Eise uber winternden, luftatmenden Wasserinsekten, besonders der Wasserkafer und Wasser wanzen." It seems that the whole question of respiration beneath the ice is one which has not yet been fully investigated.

The longevity of the adults is yet a question. It would be a

difficult matter to determine in nature because of the character of their habitat. They evidently migrate from pond to pond as food supply, water, seasonal differences, and other conditions require. In the laboratory it has been difficult to keep them alive for more than a few weeks, even in winter, and they certainly live as adults through the winter months in the ponds. Repeated efforts may solve the difficulty.

Of their habits of flight the following observations may be of interest. On February 9, 1924, when observing living specimens in the laboratory at night with electric light, two individuals flew out toward the light and fell upon the table showing that they are positively phototropic at times.

Other evidences of phototropism are many. Mr. Robert Guntert says he has taken them many times around the electric lights at night when he was collecting. On March 13, about 8 a. m., one *Buenoa* flew toward the window from an aquarium sitting on the window sill. Sometimes when brought into the laboratory in the evening and spread from the "live bottle" upon a piece of toweling paper to dry, some of them would fly to the windows or electric lights. Most of these incidents happened in March and April, and when the days were moderate in temperature.

April 15 while collecting at Smith's pond several individuals were seen to take flight. Evidently the weather conditions were favorable, for it was a beautiful, quiet morning, and they were migrating. Some were also seen to alight in the pond. Sometimes they had difficulty in getting their wings adjusted properly immediately after alighting, and would kick about and dive in the water rapidly trying to detach themselves from the large air bubbles which hung beneath the hemelytra. They seem to strike the water at a low angle, back upward, and immediately turn over as in the normal swimming position. The pearly-colored hemelytra evidently serve to disguise them from above while in flight and from below while in the water so their enemies may not so easily see them. On taking flight from the water they swim close to the surface for a short time and then suddenly shoot forth from the water at about a thirty-degree angle and take wing. *Notonecta* was observed flying about two weeks earlier. They sometimes climbed out on sticks or other objects near the shore, waited till the hemelytra were dried for a few seconds and then took wing. Other times they would start immediately from the water, fly a short distance, fall back into the water and float along on the surface for a little time dorsum

upward and then fly away. *Notonecta* is mentioned here simply in comparison with *Buenoa*.

Of the food habits of *Buenoa,* they are said to feed largely upon *Entomostraca,* and occasionally on other forms, which they hold in the crib formed with the front pairs of legs and pierce with their stylets. About February 9, 1924, while dissecting the abdomen to see if eggs were developing, it was noticed that the stomach contents were green in color. The material was examined under the microscope and gave all the appearances of being the green chlorophyll particles of *Spirogyra,* some of which was contained in the aquarium jar from which the bug was taken. Two or three other individuals within the next two or three days were seen to contain the same material, but not in such quantity as the first one. One bug was found dead in the water still holding a thread of *Spirogyra.* Part of the cells of the thread did not contain their normal amount of chlorophyll, but the beak was not inserted in any of them. It may have just gotten tangled in the thread while in its death struggles. It may be that predacious *Buenoa* also feeds upon algæ as does its relative, the corixid, when other food is not available.

It is worthy of note in connection with the study of the hæmoglobin cells discussed in the following pages that the swimming habits vary. After the bug has been to the surface for air (it seldom remains at the surface more than two or three seconds, which is quite opposite to the facts with *Notonecta* or other water bugs) it makes quick vigorous strokes in order to keep itself beneath the surface. These strokes become less and less vigorous until it may rest in the water in almost perfect equilibrium, sometimes for several minutes. Then it begins slowly to sink and has to use effort to sustain itself. It is usually not long after this till the bug returns to the surface for a fresh air supply. The *Notonecta* and other water insects seem not to be able to remain beneath the surface as long as *Buenoa* and keep their activities the same. Either they swim more vigorously about and come to the surface oftener for air, or they take down a good supply of air and attach themselves, with no activity, to some object on the bottom, or hang at the surface film in constant contact with the atmosphere. Fernald's Applied Entomology, page 184, says the corixids "are able to remain under water without coming up for air for a much longer time than the backswimmers," referring to the Notonectidæ as a group. This has not been found to apply to *Buenoa,* except when the corixids grab hold of something below the surface and remain there motionless. In that case they would

not use nearly so much air or oxygen as the *Buenoa* which are gliding about in the water constantly.

In the ponds on a windy day, when the waves are large, *Buenoa* usually swims in the quieter parts where surface contacts are more easily made. They were often seen hovering about the piling of the bridge, where they were collected when they could not be located elsewhere. At the same time *Notonecta* might be found easily about the edges of the pond in the quite rough water.

It has been observed that *Buenoa elegans* is usually found in the shallower parts close to shore instead of out in the open and deep waters, as is so often the case with *Buenoa margaritacea*. They both are found usually in the same pond, and it may be that the smaller species stays closer to shore for protection from the larger one, which may attack it on occasions when they meet.

The *Buenoa*, as a group, appear to be able to swim almost as fast and as well as does *Notonecta*, although it is much smaller. This may be due to their larger development of muscles in the metathorax.

All the Notonectidæ, and especially the *Buenoa*, have a remarkably well developed stream line form, as has been discussed by various writers. This, with the oarlike hind limbs, makes them very well adapted for living in the water.

SOUND DEVICES.

In the University of Kansas Science Bulletin, Volume XI, pages 196, 197, is a description of the sound devices of the male of the species *B. margaritacea* in the following words:

"Upon attaining the adult stage a sexual dimorphism becomes apparent. Besides the structural differences of the genitalia, the anterior legs of the male possess on the inner faces of the femora and the tibiæ peculiar stridular areas.

"The tibial structure is borne on the inner face of a prominence, which is formed by the elevation of the inner angle or margin of the tibia near its base into a thin but elongate spur. This spur is lacking in the female, and not discovered in the nymphs. (See Pl. XXIV, Figs. 5 and 6.)"

Again on page 195 is this:

"The males possess stridular areas upon the inner faces of the femora and tibiæ, and also on the sides of the face at the base of the beak. These are shown in the drawings on plate XXIV. The tibial structure is borne on the inner face of a spur near the base of the anterior tibia. When the fore limbs are brought up to the head, it will be seen that the stridular areas of the limbs meet those on the base of the beak. (See the figure on Pl. LIV.) The production of sound is doubtless the purpose of these structures. European workers have recorded notonectids as capable of producing chirping sounds.

Mial quotes Redfern to the effect that *Notonecta* makes a noise like the word 'chew' repeated three times."

In a lecture before the Entomology Club at the University of Kansas in the autumn of 1923 Doctor Hungerford told of hearing *Buenoa* chirping in an aquarium in the laboratory at Douglas Lake, Michigan, the preceding summer, and also described the accompanying behavior of the sexes. His description indicated that a few days before that date *Buenoa* was heard chirping in the laboratory here, but the source of the sound was not then recognized. In the laboratory, one evening in September, peculiar noises were heard coming from a galvanized iron aquarium where some live adults were kept for study. The noise was rather faint, so cautious approach was made and the light turned on to see what was happening. Before the place was reached and before the light was turned on, the noise ceased and did not occur again during the evening. Other lights were on in the laboratory at the time. The noise sounded something like drawing a nail quickly across the teeth of a rubber comb. Nothing unusual was seen in the actions of the bugs swimming in the water, and it was thought to be a mistake until Doctor Hungerford gave his description. It is a common occurrence for them to make considerable noise by butting their heads against the sides of the aquarium, and this might be mistaken for chirping by one who has not become accustomed to them. Constant watching since that time to detect any further sound production or mating has failed.

It should be added that upon careful examination it is found there is also a strong but minute spurlike peg on the outer lateral surface of the anterior coxa which aids, no doubt, in sound production. There are also a number of strong setæ and a chitinous thickening at the distal end and inner lateral surfaces of the anterior femora which are probably used to produce sound by plucking on a chitinous-thickened corner on the sides of the face just below the antennæ. The females possess this last device, also.

So it appears that *Buenoa* has, in the case of the males, three pairs of sound devices and in the female one pair. Beginning at the cephalic end of the body, in the male the first pair is the chitinous thickening and setæ at the distal end and inner lateral surfaces of the front femora, together with the chitinous-thickened sharp corner on the sides of the face just below the antennæ. This is also the one pair for the females. The second pair for the males is the tibial structures borne on the inner face of a prominence near

the proximal end of the prothoracic tibia, together with the areas on the lateral faces of the prongs near the base of the beak. The third pair consists of a washboardlike area about midway between the ends on the inner lateral surfaces of the anterior femora, together with a spurlike seta near the base and on the outer lateral surfaces of the anterior coxæ. There are some exceptions to the above rule for some of the *Buenoa*, but it is true in most cases. With all these sound devices it appears they may have a language all their own with quite a variety of sounds and combinations, especially in the case of the males. For an illustration of these devices in their relative positions, see plate LI, figure 4.

ECONOMIC IMPORTANCE.

Fernald in Applied Entomology, page 184, says of the Notonectidæ: "They are carnivorous, feeding on other small insects, but are of little importance." Since the discovery of the hæmoglobin cells in *Buenoa*, they may appear of considerable scientific importance. Their economic importance is not so certain, but they feed upon the small crustacean life of the waters as do some fish, and *Notonecta* has been said to attack small fish. They may be of much more importance to fish culture than is usually thought.

COLLECTING NOTES.

Most of the materials collected for these studies were taken at the Union Pacific railroad bridge mentioned on page 269. It was found to be the most convenient because it was a permanent pond and the bugs could be found there at any time when fresh materials were desired. Most of the studies on *B. margaritacea* were done with fresh material. The piling at the foot of the bridge have cross timbers bolted to them just at the surface of the water. These were found to be very convenient because *Buenoa* usually swims in deep water and well out from the shore. One could walk out on these cross timbers where the water was fifteen or twenty feet deep or stay nearer the bank, as he chose, and still have considerable area of water over which to collect. In this position it was not difficult to see the bugs balancing themselves some distance beneath the surface and to quietly slip the net beneath them and bring them to the surface.

Other collecting was done at Stubb's pond, nearer town, at the brick-plant pool, at Smith's pond east of Lawrence, and in different counties of central Kansas during the month of June, 1923. Some-

times quite a number of *Buenoa* would be taken in the water contained in a cow track in a pool that was drying up. Shallow pools often contained more of them than larger and deeper ones. They seemed to be found during the summer months in the shallow pools where crustacean life was most abundant more than in the deeper ones.

A common water net at the end of a broom handle was used in most cases. The live material was brought into the laboratory in an empty bottle containing a few grass blades, leaves or cellucotton to take up the excess water. If, as is usually well known, one should attempt to bring them to the laboratory in water they would soon drown. When drowned they could, like young chickens caught in a thundershower, be revived by drying out. Often, if any of them were sticking in a little water at the bottom of a bottle, it was found best to empty them out on toweling paper and let them dry till they hopped about vigorously before putting them into the aquarium jar. The eggs and nymphs were sometimes placed directly into the fixing fluid for sectioning or other study when taken from the water. The following fixing fluids were used: for the adults and nymphs: picroformal, picrosulphuric acid and 85 per cent alcohol; for the eggs; picrosulphuric acid, and Perenyi's fluid of chromonitric acid.

TECHNIQUE.

MOUNTING AND DISSECTION.

The technique used in the work of mounting and dissection for the grosser morphological studies was suggested by R. H. Smith in the 1923 September number of the Annals of the Entomological Society of America. To one with no experience the task of carefully dissecting a bug 7 to 7.5 mm., or slightly more than one-fourth inch long, is a rather difficult one. It may be of value to some one to describe here the methods used.

At first the attempt was made to pin down the creature upon parafin in a large dissecting dish, such as was used in the elementary classes in morphology. The pins were too large and in the way of the dissecting needles. They did not hold well and would tear out at the sides and mutilate the specimen. Small "minuten nadlen" were then tried in a much smaller dish of paraffin and one which did not require nearly so much salt solution. The making of quantities of physiological salt solution every little while was somewhat of a nuisance. It had to be used because the hæmoglobin cells and other tissues would hæmolyze and so cloud the medium in which one was

working that nothing could be seen clearly. The red coloring substance of the hæmoglobin cells was responsible for most of the difficulty in keeping a clear field. The "minuten nadlen" did not prove satisfactory, for they would not hold well in the paraffin, and gave the same difficulty of cutting out at the sides of the specimen as did the larger pins. In casting about for some better method the article by Mr. Smith was found.

By this method and for this purpose it has been found better to use a small dissecting dish with a lid to keep the salt solution from evaporating and ruining the specimen when interrupted by class work or other difficulty. A little tin box with a lid, about three inches in diameter and one inch deep, was filled nearly half full with melted paraffin. This was allowed to cool. The bug was then held in one hand with a small pair of tweezers by the legs or otherwise depending upon the position desired for placing and the ease of holding. In the other hand was held a piece of wire of the size of bailing wire or a little larger. The wire was placed in a gas flame till hot and then used to melt a small area in the smooth surface of the paraffin near the center of the dish. Immediately the bug was placed in the melted area in the position desired. The paraffin hardened quickly as it cooled and held the bug firmly in the desired position. If a film of water happened to be clinging to the bug, a second application of the hot wire around the bug was sometimes necessary to make the paraffin hold well. A large needle might just as well have been used to melt the paraffin. To hasten the cooling and hardening of the paraffin and to prevent cooking of the more delicate tissues the physiological salt solution was poured immediately into the dish but not directly on the bug, for sometimes it would wash away the melted paraffin around it. At this stage all was ready for the dissection to begin.

Instead of paraffin in the dissecting vessel, beeswax and shoemaker's wax were also tried. Beeswax was almost as good as paraffin but a little soft. Shoemaker's wax being very dark in some cases was an advantage because of the contrast with the insect tissues. Usually it remained too soft, or the heat from the electric light used was sufficient to soften it so that it did not hold the insect satisfactorily.

A medium sized sewing needle mounted in a handle, heated in a gas flame, flattened on an anvil, and sharpened with a fine carborundum or razor hone under the binocular to a delicate arrow-shaped point, was used to open the insect. The opening was done by pierc-

ing the body integument and then cutting outward against the paraffin. Otherwise the internal tissues would be crushed and mashed out of place. The chitinous body wall of *Buenoa* is quite thick in places considering the size of its body. After a cut was made entirely around the body and outward against the paraffin the top section could be lifted off and the interior examined in its natural position. In dissecting the tougher portions it was found sometimes to be of advantage, in getting the insect thoroughly fastened down in the paraffin, to clip off the wings close to the thorax, or in other cases to clip off the legs.

The scalpel made from a needle just described; two or three dissecting needles made of "minuten nadlen," mounted in handles and bent at the tip or sharpened to suit the worker; one or two dissecting needles from sewing needles of the medium sizes, mounted and sharpened; two ink droppers or pipettes, one with a large opening and the other with a smaller one, to remove bits of tissue suspended in salt solution for microscopic examinations; a few slides and cover slips; a large binocular microscope with an oil immersion lens attached; and a smaller binocular, were the tools used in this study. Also a projection microscope was sometimes used.

TRACHEAL TECHNIQUE.

The hæmoglobin cells studied are closely associated with the tracheal system, and it was therefore necessary to trace out something of the structure of this system. A study of the tracheal system was also necessary to aid the findings of the other morphological studies. For this purpose it was found to be much better to use fresh specimens in which the tracheæ were yet filled with air, giving them a silvery white lustre and making them visible. It was almost impossible to distinguish tracheæ from other tissue when once filled with water. One could not work long with the same specimen till the larger tracheæ would become so filled. This was especially true with those of the thorax. Some of the suspected connections could not be clearly traced, and it was decided to try a process of injection of the tracheæ described by August Krogh (45), of Copenhagen University, in 1917.

For an injection fluid he used a mixture of paraffin, beeswax, colophonium and turpentine, stained as deeply as possible with alkanna. It was difficult to get any alkanna, and that which was finally secured may not have been of much value. The process described by Krogh was repeatedly tried with *Buenoa* and found unsuccessful.

The substances penetrated the other tissues as well as the tracheæ and made the tracheæ invisible. The process may be good for the larger insects.

Glycerin agar melted and colored deeply with finely powdered carmine was tried instead of the mixture above described by Krogh. It worked very poorly. The process of attempted injection used for this was the same as is given on page 285 except that the colored agar was placed in the large bottle and cooled till it hardened.

The bugs, inclosed in a wire-screen cage so they would not sink, were placed in a bottle on top of the solid agar. The air was exhausted and the bottle containing the colored agar and the bugs was immersed in a hot-water bath until the agar melted and the bugs sank into it. The air was then slowly readmitted to force the liquid into the tracheæ. With the reduced air pressure the substance would boil at a very low temperature soon after it melted and so disrupt the tissues. But the process did not work well even when boiling was prevented.

Krogh also suggested using colored turpentine instead of the above mixture in the case of animals with closely intact spiracles. This was tried with considerable success, but no substance was found which would satisfactorily color the turpentine so that the tracheæ would stand out in strong contrast to the other tissues. Eosin, powdered carmine, picric acid, methylene blue, and borax carmine solution were used in attempting to color the turpentine. At best only a weak stain, which under the binocular was greatly diluted, was secured with any of them. A substance of a lipoid nature was necessary according to Krogh in order to penetrate the tracheæ. Turpentine was such, but was not strongly enough colored.

It was decided to try Grenacher's borax carmine, a substance which has a very dark red color. It was felt that this surely would give the desired contrast if it could be made to penetrate deeply enough. It penetrated very nicely to the remote tips of the tracheæ, giving a bright-red color under the binocular in strong contrast to the surrounding tissues. A large part of the sketches of the tracheal system of *Buenoa* as figured in the accompanying plates were drawn with the aid of this injection material. It can no doubt be as successfully used with other minute or small insects. A difficulty arises when an attempt is made to use it with the larger insects or larvæ. An injection was made of a nearly mature tipulid larvæ, but it was found that the fluid would flow out from the larger tracheæ when the insect was handled.

The article by Krogh may not be readily available to the reader, as it was not in our case, so there is given here something of a repetition of the device used by him, together with the modifications as used in this laboratory. A quantity of the injection mixture, Grenacher's borax carmine, was kept in a wide-mouthed bottle of about 250 cc. capacity with a ground tubulated stopper. If the ground tubulated stopper was not available, a large rubber stopper with a piece of glass tubing inserted was used. The neck of the bottle was reënforced with adhesive tape to prevent its being easily broken. The diameter of the rubber stopper was increased with the same material to prevent its being pushed too far into the bottle by the air pressure. The animals to be injected were removed from the aquarium jar to a piece of toweling paper till the film of water was removed from them. They were then deeply narcotized by dropping them into a large bottle with a wad of paper or other material saturated with a few drops of ether and replacing the stopper till they were quiet for a moment. This was done to make them immobile and render ineffective the mechanism for closing the spiracles or tracheæ.

A number of the bugs were usually so treated at the same time, and one kept for a control to see that all were not killed with the ether. The others were then placed in a wire-screen cage in the bottle with the injection mixture, but kept above it till after the air was exhausted. The stopper was then put in place and the bottle connected by means of heavy rubber tubing to an air pump or aspirator. Previously the aspirator was fastened to the faucet at the sink, one screw clip closed and the other screw clip opened. The water was then turned on at the faucet and the air exhausted from the bottle containing the bugs. After fifteen or twenty minutes the exhaustion of the air was usually sufficient, for a pretty good vacuum could be secured by such means. The cage containing the bugs was then turned over by turning the bottle, so that the bugs were immersed in the liquid; one screw clip was closed, and the second screw clip was slowly opened till air bubbled through the wash bottle used to indicate the rate of release of air into the vacuum. This was so regulated that the air bubbled in quite slowly. A thermometer tube or other tube with a bore of 0.1 mm. or less was used to aid in regulating the air supply as was suggested by Krogh. The joining was made with heavy rubber tubing and all connections were made airtight with a little vaseline.

The most important point in the whole technique was the slow in-

crease of the pressure which secured a complete filling of the tracheal system. After about half an hour the second clip was opened a little, care being taken that the injection should never be completed in much less than an hour. When the pressure had risen to normal the bottle was disconnected and opened, and the bugs taken out and mounted in the paraffin dissecting dish previously described.

CELLULAR TECHNIQUE.

The technique used for the cellular and more minute structures was the same as is ordinarily used for such work. The tissues were fixed and embedded in paraffin, sectioned, mounted and studied under low-, high- and oil immersion lenses with a binocular compound Bausch-Lomb microscope equipped with an Abbe condenser. Attempt was made to use the projection microscope, but the lighting was insufficient under high magnification.

EXTERNAL MORPHOLOGY.

Concerning the external morphology of the Notonectidæ, and especially about the genus *Buenoa,* little seems to have been written or figured. From the viewpoint of pure morphological interest and from a consideration of the possibility of aiding in determining the phylogenetic or other relations of the order Hemiptera-Heteroptera, a thorough study of the morphology of *Buenoa* and the other genera of the Notonectidæ might be of considerable value. No pretense of such a thorough study is made in this paper. What is given may help further study. The terminology used is intended to be in accordance with that of the latest authorities. However, Crampton, 1916, wrote that the Hemiptera-Heteroptera were composed of four typical groups or suborders, and he called the notonectid group the Euhemiptera or true Hemiptera. He said that, "The true Hemiptera are so highly specialized that they are of no great interest from the standpoint of the study of phylogeny; but the Homoptera give some valuable hints as to the relationships of certain of the higher forms." (Ent. News, XXVII: 301.)

Ashton, 1837, in a discussion of the wings of Hemiptera, figured and described the union of the wings of *Notonecta.* Berlese, 1909, in "Gli Insetti," in that part of the volume dealing with external morphology, figured the abdomen and genitalia of *Notonecta glauca.* Hoppe, 1911, in "Die Atmung von *Notonecta glauca,*" gave some descriptions and a few figures of both adults and nymphs. Rich,

1918, in describing the respiratory organs of a notonectid in South Africa, which he was not able to identify, gave two figures of the abdomen. Hungerford, 1919, in the University of Kansas Science Bulletin, gives a number of plates showing something of the adults and nymphs of the three genera, *Notonecta, Buenoa,* and *Plea.* Other figures have since been given of *B. macrotibialis* and *B. limnocastoris.*

HEAD.

The head of *Buenoa* appears as in Plates XLIV and XLIX. The eyes are quite large in comparison with the size of the head and rather close together. They approach closest at the notocephalic margins of synthlipsis. In the species like *B. margaritacea,* they form, with the lines of the prothorax, a smooth contour as seen from a dorsal view. In those species more of the type of *B. elegans* they project laterally or appear to protrude beyond the lines of the prothorax when viewed dorsally. The ommatidia are quite regularly hexagonal. The epicranium or sclerite between the eyes is narrow and extends from near the ventral separation of the mesal or inner margins of the compound eyes to the caudal and dorsal part of the head, and seems to form the dorsal margin of the occipital foramen. The genæ form the more lateral and ventral parts of the occipital foramen. The frons is not distinctly separate from the epicranium in the adults, but appears more so in the nymphs. The clypeus seems to be fused with the frons. The postgenæ are small and linear. The genæ are rather long and have a part beneath what appears to be the beak. The trochantin of the mandible or basimandibula is rectangular and has the angle in front of the antennæ large and sharp, and is evidently used to produce sound by plucking with the front femur as described on page 278. The labrum is a small flap distinctly seen below the clypeus, and is covered with many fine setæ which makes it appear like a moustache. (See Pl. LI, Fig. 4.) The labium with four distinct segments has a ventral groove in which are contained the stylets consisting of a pair of mandibles and a pair of maxillæ. The proximal segment in the females is considerably enlarged sternally. The part of the head to which it is attached is correspondingly large, probably to better accommodate the larger salivary glands that are present in the males. The proximal segment in the males is rather inconspicuous. The second segment in the males is prolonged laterally into prongs. On the lateral faces of these prongs are washboardlike areas used in the production

of sound. Along the median line of these areas is usually a row of very small but darkly chitinized toothlike setæ which evidently aid in the process. The third and fourth segments of the beak are longer, and all are open ventrally with a narrow groove. Near the base of the beak and beneath the labrum the mandibles are seen. They often protrude at the end of the beak in mounted specimens or live adults held under the binocular can be seen to make them protrude quite often. The mandibles and maxillæ are rather loosely held together in the sheath of the labium. The mandibles have a row of serrate recurrent spines on the lateral surfaces at the tip. The mandibles and maxillæ form the tube through which the juices foods are taken. Time has not been taken to work out their exact relations and form.

THORAX.

PROTHORAX. The prothorax appears to consist of an almost continuous segmental ring of chitin, longer in width laterally than in length in the caudo-cephalic direction. Some evidence of sutures is seen at the ventral parts to mark off the sternite and the antecoxal pieces, but the pleurites appear to have wholly fused with the surrounding sclerites.

MESOTHORAX. The mesothorax is more complicated than the prothorax because it bears the front wings or hemelytra. The dorsum of the mesothorax extends well cephalad under the dorsum of the prothorax and appears to be divided at the anterior margin into pairs of sclerites by three sutures. One extends caudad and dorsad on either side from the anterior and caudo-lateral margin. (See Pl. XLIX, Fig. 2, dotted lines.) These sutures mark the dividing point of two sets of large muscles in the mesothorax. The sternum appears as a broad central plate ventrally between the mesothoracic legs. A suture marks off the mesal margin of the antecoxal pieces as shown in the figures. (Pl. XLIX, Fig. 1.) The pleurites appear as shown in figure 3, plate L.

An interesting pair of sclerites, which appear at first to be antecoxal pieces of the metathoracic legs, are two large ones called by Rich, 1918, extensions of the mesothoracic scutellum. These appear to arise from an episternite of the mesothorax, and extend caudad and ventrad, covering almost entirely the metathoracic coxæ and a large part of the metasternum. They cover and help with their fringes of setaceous hairs to form the ventral air channels to the thoracic spiracles. This fringe of hairs is on the mesal and caudal

edges only in the adults, but appears also on the outer lateral edges in the nymphs, as is shown for the fifth-instar nymphs in plate LVII, figure 1 and 2.

The sclerites about the base of the front wing have not been worked out thoroughly. The caudal margin of the mesothorax is joined broadly and firmly to the anterior margin of the metathorax.

SPIRACLE. On the cephalic margin of the mesothorax, laterally and ventrally beneath the caudal margins of the prothorax, and in the more or less nonchitinous membrane connecting the prothoracic and mesothoracic segments, are the spiracles. They exist simply as a rather large opening in the membrane, and seem not to have any sclerites about them such as the peritremes of the abdominal segments. The insect seems to be able to close them in case of need by simply pulling the prothorax and mesothorax closer together, so that the posterior and anterior margins respectively telescope one another more closely. These spiracles appear to be the largest and supply the biggest and most remarkable array of tracheæ in the whole body. This bunch of tracheæ is no doubt so developed because it supplies the air to the wing muscles. (Pl. XLIV, *TP.*)

METATHORAX. The metathorax is the largest segment of the entire body. This must be because it contains the muscles of the hind wings most used in flight, and also those of the swimming legs which in the water are almost in constant use. The metathorax of *Buenoa* is relatively much larger and projects further caudad on the dorsal side than it does in the *Notonecta*. This may be due to the more constant use of the swimming muscles in *Buenoa* than in *Notonecta*.

The exterior surface of the metathorax appears smoother than that of the mesothorax. The metathoracic tergite is divided on the dorsal median line by an internal projection or apodeme. This is the largest sclerite of the body. The sclerites about the base of the wing are rather small and undifferentiated. The pleurites appear large and distinct. (See Pl. L, Figs. 3 and 4.) The metasternum is a large ventral plate and possesses a large spinelike projection extending caudad on the mesal line between the coxæ of the hind legs. (See Pl. XLIV and XLIX, Fig. 1.)

At the cephalic margin and from the dorsal surface there extends internally and ventrally, about two-thirds the diameter of the body, a large apodeme which apparently serves as a fastening for the muscles supplying the wings of both thoracic segments. This apodeme pushes the dorsal aorta and œsophagus to a quite ventral

position in this region of the body. Another similar apodeme at the caudal margin of the metathorax does likewise, except that it does not extend so far ventrally, and extends cephalad and laterad at the same angle as is formed by the caudo-lateral margins of the metathorax to which it is also joined. (See Pl. XLIV, *IC* and *Ic.*) Prominent ventral apodemes are also present which somewhat surround the ventral muscles leading to the legs. (See Pl. XLVI.)

The spiracle, with a somewhat hidden chitinous opening, is just beneath the base of the wing. (See Pl. L, Figs. 3 and 4.)

WINGS.

The wings are figured in plate L, figures 1 and 2. For the fifth-instar nymphs, see plate LVII. Both wings are quite transparent, but the proximal area of the fore wing is more translucent. The wing venation is much reduced and quite difficult to determine accurately. The labeling given is approximate.

The fore wing has two unique devices for fastening to the body when not in use. Such or similar devices have been figured for *Notonecta* by Ashton, 1837. The snaplike device at *L* in figure 1, plate L, fits into a sort of hook at *L* in figure 3 of the same plate. The snaplike device at *B* in the figure fits into another groove or hook at the median dorsal line of the body. The membranous part of the wing at the apex is quite coneshaped in life. In some species this part of the wing may aid in breaking the surface film when the insect comes to the surface of the water for air. In most of the *Buenoa* studied it does not do so. The film is usually broken by the abdominal hairs.

The hind wing is quite delicate and beautifully iridescent. It is folded in two folds beneath the hemelytra or fore wing. The veins of the region near the front margin are the only ones that are distinctly visible unless the wing is mounted dry. The mounting of this wing is a rather difficult matter if one desires a perfect specimen.

LEGS.

The legs of the thorax have each the same number of segments but are variously modified to suit the purposes for which they are adapted. All have the common segments, coxa, trochanter, femur tibia, two tarsal segments, and two tarsal claws at the end of the distal tarsal segment. (See Pl. XLIV.)

The legs of the prothorax and the mesothorax are much alike in form, but those of the mesothorax have somewhat longer and more

slender segments than those of the prothorax. Many setaceous spines are so arranged that when the legs are flexed they form a crib in which to catch and hold prey. These spines may also aid in forming the ventral air chamber. They are lined in definite rows, especially on the tibiæ and femora. (See Pls. XLIV and LI, Fig. 6.) On the front legs are the modifications for the production of sound described on page 278 and again on page 287. (See Pl. LI, Fig. 4.) In this figure corresponding numbers are given to those parts which seem to work together in the production of sound. Their relative positions are the bases for the conclusions. The coxæ of the two front pairs of legs are quite long and ventrally distinctly visible. The trochanter is rather short and V-shaped, with a number of setaceous hairs or spines on the apical surfaces which help form the ventral air chamber of the thorax.

The hind legs are especially modified for swimming, as they have a fringe of setaceous hairs on each side of the segments including the femur to the tarsal claws. The fringe on the femur is not strongly evident but it is well developed on the tibia and tarsal segments. The whole leg bears a strong resemblance to an oar. In the first-instar nymph the fringe is found only on the tarsal segment. The tarsal claws of the adult are of the same length at the tip of the tarsus as the swimming fringe and are much reduced and inconspicuous. In the nymphs they are much more conspicuous.

THE ABDOMEN.

GENERAL. Eleven segments are found in the abdomen. The first two caudad of the thorax are not distinctly separated, but are firmly joined together and to the caudal margin of the metathorax so far as the tergites and sternites are concerned. The pleurites are somewhat more distinct and more easily separated. Each segment from the second one to the seventh, inclusive, is made up of a single tergite, two pairs of pleurites, and a single sternite. (See Pl. L, Fig. 4; and Pl. XLIX, Fig. 1.) In the seventh segment there is apparently an extra pleurite on each side, making three pairs of pleurites for this segment. (See Pl. LV, Figs. 4, 5 and 8; and Pl. LII, Figs. 8, 9 and 12.) Caudad of that the pleurites are simply chitinous membranes. The genital segments are left for later description.

HAIRY FRINGE. Near the juncture between the two pleurites on either side of each segment, and extending cephalad from the seventh segment to the thorax, is a row of long, blackish, setaceous, aquifuge hairs which fold closely parallel to each other and mesad, forming

the abdominal air channels. The tips of these hairs meet those from the opposite side in the cephalic part of the abdomen. Near the caudal tip they meet with shorter hairs on the ventral median carina. (See Pl. LII, Fig. 12, for the male; and Pl. LV, Fig. 8, for the female.) They are also figured on other plates, but are not usually drawn as long as they appear in life, because if they were one would not be able to distinguish other parts in the drawings. Under magnification these hairs are found to be nearly flat. Together with the surface tension of the air bubble which they hold they are able to keep water out of the spiracles. The surfaces of the air chambers also seem to be aquifuge. At the caudal tip of the abdomen this air chamber is divided by the median carina into two separate parts, but these parts unite shortly cephalad and continue as one to the head on the ventral surface of the body.

SEVENTH STERNITE. The seventh abdominal sternite in the females is projected caudally into a sharp V-shaped point on the mesal line. The function in oviposition of this sclerite has been described on page 271. It also serves as a ventral shield to the genital parts at the tip of the abdomen, and may aid with the abdominal hairs in breaking the surface film when the animal comes to the surface for air. (See Pls. XLIX, L, LV and LVII.)

FIFTH STERNITE. The fifth abdominal sternite in the adult is especially large. It projects forward on the median ventral line until it almost divides the fourth sternite in two halves. (See Pl. XLIX, Fig. 1; and Pl. L, Fig. 4.)

FOURTH STERNITE AREA. Just mesad and caudad of the fourth abdominal spiracle, and on the fourth sternite, is a smooth, rather glandular-appearing area. It is quite noticeable ventrally in both males and females when the fringe of hairs forming the ventral air chamber is turned aside. Examined under high power it is found that the smooth surface is due to the fact that there are no setæ on the sclerite at that place. The entire inner surface of the air chamber next the body, except the peritremes around the spiracles and the area just mentioned, is covered with quite an even distribution of minute setæ. They seem to aid in making the surface aquifuge. Just what function this smooth area may have is not known. It was closely examined for glandular openings, but none were found.

FIRST TO THIRD STERNITES. It has been written that the first and second sternites were closely united. Berlese figures those for *Notonecta glauca*. He shows the first sternite to have internal projections or apodemes, but otherwise to be quite small. *Buenoa* appears

to have the first sternite quite large, extending forward and laterally to a point near the middle of the methathorax. Immediately on each side of the median ventral line, and projecting internally and caudally from the first sternite, are two darkly chitinized and quite long apodemes. The caudal extremities of these processes extend into the third or fourth abdominal segments. Muscles which appear to aid the hind legs in swimming are attached to them. The second sternite, closely united to the first, is a narrow sclerite immediately caudad of the first, and extends to the large, second abdominal spiracle at its anterior lateral corners. The third sternite, immediately caudad of this, is narrow and is the first anterior one of the venter to be colored darkly.

SPIRACLES. The spiracles are at the lateral edges of the sternites. All are quite large compared with those of *Notonecta*. Each one, except the first and eighth, are distinctly marked by the white color of the peritremes, in strong contrast to the blackish color of the sclerites which surround them. Those of the second and fifth segments are the largest. The first abdominal spiracle is so far forward that it appears superficially to be at the middle of the metathorax. (See Pl. L, Fig. 3; and Pl. XLIV.) The eighth one does not appear except in the nymphs. (See Pl. LVII, Figs. 1-4.) In the adult it has atrophied because of the retraction of the segments caudad of the seventh. It may be rather nonfunctional in the nymphs.

SWIMMING HAIRS. In both male and female a row of long, silky, setaceous, free-swimming hairs is found near the junction of the seventh tergite with the pleurites. This row usually extends cephalad on to the same part of the sixth segment. (See Pl. LII, LV, and LVII.) The function of these hairs seems to be to aid in balancing the bug when at the surface film for air. When it is in that position these hairs appear to attach themselves to the film, and serve as anchors. They also may help some in swimming, serving somewhat as rudders or like the tail fins of a fish.

DORSAL SPINE. In the adult male, on the sinistro-caudal margin of the seventh tergite, is a long and often quite black spine. The approximate parts of the tergite are so modified as usually to make the whole segment somewhat unsymmetrical. A corresponding spine or "strigillum" is found on the males of the corixids, but in that family the adjoining sclerites are much more unsymmetrical. This structure seems to be another indication of some relationship between the

Notonectidæ and the Corixidæ. Whether a similar spine is ever found in *Notonecta,* or the other genera of that family, has not been determined. The purpose of this spine has not yet been learned. It is supposed that it is of some use in mating. It appears to have nothing to do with holding the wings in position or other such use, and is not found in the females. The males of every species of *Buenoa* studied possess it. (See Pl. LII, Figs. 9 and 11; and Pls. LIII and LIV.)

HEART AREA. On the dorsal median line of the second to the fifth abdominal tergites, inclusive, is a very thinly-chitinized membranous area. This area almost divides the tergites of these segments into two halves. If a live adult bug is held in position with the dorsum upward under a high-power binocular regular pulsating movements can be seen. These movements are caused by the dorsal vessel, or heart. An enlarged portion, and probably the most functional part of the heart, is located there. The membranous portions of the tergites there allow more expansion and freer movements of that organ. (See Pls. XLIV and XLIX, Fig. 2.)

EXTERNAL GLAND OPENINGS OF THE MALE. In some species of *Buenoa, B. margaritacea* and those of similar size and appearance the males possess near what seems to be the junction of the pleurites of the first and second abdominal segments, on each side, a pair of small, blackish, glandular-appearing spots. These spots are also underneath the edges of the hemelytra when closed and beneath the outer lateral edges of the large shieldlike plates over the bases of the metathoracic coxæ, but are still plainly visible from the exterior. In one or two species, probably not yet described, similar spots are found which are not black. The spots are distinctly visible to the naked eye, vary somewhat in size, but are about one-half millimeter in diameter. When viewed vertically with magnification they have somewhat the appearance of a rather large, thick doughnut with a small hole in the center. The hole is probably the opening for the discharge of some fluid upon the surface of the body from what appears to be a gland underneath. The colored part at the surface of the body around the opening consists of many small, very darkly colored or chitinized cells. Most of the cells are very perfectly hexagonal in form. The thinly membranous chitin surrounding the organ enables one to easily pluck it out. The outer colored portion is quite hard and projects a little way above the surface. When plucked out a string of tissue clings to it somewhat as shown in

figure 1, plate LI. This distinctly visible glandular opening, visible although somewhat covered at times with translucent wings, may prove of considerable taxonomic value aside from its morphological interest. The function is not known. It may be a scent gland for the attraction of the females, or it may provide the oily, aquifuge substance covering the interior of the ventral air chamber. (See Pl. L, Fig. 3*G;* and Pl. LI, Figs. 1 and 2.)

The suggestion may occur that this structure is a modified spiracle, but all the spiracles have been accounted for, one each in the mesothorax and metathorax and the first eight segments of the abdomen. Sectioning has not yet been successful enough to give any further information about it.

GENITAL SEGMENTS.

TERGITES. The eighth abdominal tergite is the last one which is very distinctly seen from a dorsal position. The others may all be seen in the female if properly extruded. The eighth tergite is very similar in shape and appearance in both males and females, but in the females it more closely incloses the tip of the abdomen. At the caudo-lateral edges it is prolonged somewhat into a sort of oval flap or projection which covers the more caudal segments. This projection is called by Berlese in *Notonecta glauca* the procercus. The outer surface of these projections is covered by one or two patches of small setæ. (See Pls. LII and LV.)

The ninth tergite in the female has on the median dorsal line a cluster of setaceous hairs projecting through the opening of the eighth tergite. To its anterior projections and at the lateral margins are attached the ovipositors. (See Pl. LV.) In the males the ninth tergite is almost a nonentity. It is a thin membranous piece projecting under the eighth tergite, and almost entirely invisible from the exterior. (Pl. LII.) The genital claspers apparently arise from it.

The tenth tergite is distinctly visible in both males and females. In the male it is covered dorsally with a fine, silky coat of setæ, and appears to stand up dorsally at an angle over the anal opening. In the female its outer surface is comparatively smooth, and the styli or acrostyli, so named by Berlese, seem to arise from its ventral margin.

The eleventh tergite in the female shows distinctly as a soft chitinous point at the tip of the abdomen and adjacent caudally to the tenth. It seems to be directly over the anal opening ventrally near

the junction of the tenth and eleventh segments. In the male it is almost invisible, but appears as a slight projection at the tip and beneath the tenth tergite, above the anal opening. (See Pls. LII and LV.)

STERNITES. The sternite of the eighth abdominal segment in the female is retracted within the body and dorsad of the seventh sternite. Its anterior end is opposite the anterior end of the seventh sternite. It seems to be divided into two halves, connected by a thin, almost nonchitinized membrane. (See *8* in figures of Pl. LV.) It is certain this sclerite, as shown in the figures, represents the eighth sternite, because the tracheæ leading to the nonfunctional eighth spiracle joins it at the outer lateral margin, a position homologous with the position of the spiracles in the more anterior segments. In the male the eighth abdominal sternite extends caudad from the hind margin of the seventh sternite beneath and inclosing the genital bulb, or ninth sternite, in the position analogous to that of the seventh sternite in the female.

The ninth sternite in the female seems to consist of two chitinous rods connected by an almost nonchitinous membrane and inclosed by the eighth sternite as shown at *9* in figures of plate LII. These two rodlike structures extend cephalad to the anterior margin of the eighth and seventh sternites. At the caudal end of those rods, and between them and the lateral prolongations of the ninth tergite, the ovipositors are attached. In the male the ninth sternite is very distinct, and is commonly called the genital bulb, since it forms the ventral inclosure for the genital claspers and œdagus. Below it ventrally is the eighth sternite, caudally tipped like the seventh sternite in the female. (See Pls. LII and LV.)

The tenth and eleventh sternites seem to be very weakly represented by any structure or sclerite in either male or female. Possibly the tenth sternite is represented by a small chitinous piece between and beneath the genital claspers in the male and known, in the Homoptera at least, as the connective. The development in the fifth-instar nymph seems to indicate this. In other cases the sternites are represented, if at all, by very small and thinly chitinized membranous parts. In the tenth and eleventh segments of the female they apparently are crowded to the minimum by the gonapophyses, which occupy most of the ventral position. In the male eleventh the same thinly chitinized part must be all that remains. (See Pls. LII, LV and XLIV.)

In the male the anal opening is above the œdagus and near the

extremity of the tenth tergite on the ventral side. In the female it is in a similar position between the base of the eleventh segment and the styli. (See Pls. XLIV, XLV, LII and LV.)

GONAPOPHYSES.

ADULT. Folsom, 1922, writes that "Most authorities have found that the gonapophyses belong to the segmental series, or pairs, appendages—are homadynamous with limbs—and pertain commonly to abdominal segments eight, nine and ten; though there are different views in regard to this." The results of this study seem to show the ovipositor proper to be connected with the ninth segment of the abdomen. Two other ventral pairs of structures having the appearances of being gonapophyses are caudad of the ovipositor. These are figured as *St* and *Sty* in plates XLIV, XLV, L and LV. The ovpositors are figured in the same plates and plate LVI as *Ovip.* The pair of sclerites marked *St* terminate in two minute sharp points at the caudal tip, seem to be divided but closely united along the median ventral line, and appear in the position of a sternite on the tenth segment. Because of the delicate membranous nature of the cephalic ends of these sclerites it is difficult to be certain whether or not they may not belong to the ninth segment. The third and last pair of what must be gonapophyses (for cerci are present only in the more generalized insects) are rather large, black-tipped organs, marked in the plates as *Sty.* Berlese likely would call them acrostyli, or acrocerci, and the ones immediately cephalad of them the mesostyli or mesocerci. They are covered with many fine bristlelike setæ. They appear in the adult to be attached also to the tenth segment. They are used in oviposition, as has been described. The opening of the oviduct is between and at the cephalic end of the pair of sclerites, marked *St,* which are rather nonfunctional and rudimentary.

In the male adult the gonapophyses seem to be represented only by the genital claspers and the œdagus. The claspers, which are unsymmetrical, the left or sinistral one being larger and hooked at the tip, are attached to the ninth segment, as are the ovipositors. Also, they are closely bound by tough tissue to the connective, which must be the sternite of the tenth segment. From the caudal side of the connective and beneath it the œdagus arises and extends caudad. It consists of a series of ringlike segments or rings forming a tube opening at the caudal tip of the body. The claspers account for one pair of the gonapophyses. The œdagus accounts for another. If

there are any parts which represent a third one of the gonapophyses in the adult male they have not been discovered positively.

From the studies of the adults there are two pairs of gonapophyses attached to the ninth segment and one pair attached to the tenth At least the structures indicate that, and authorities have found some variation from the rule of eight, nine and ten.

DEVELOPMENTAL STUDIES.

EXTERNAL. Some attempt was made to trace the development of the genitalia in the instars. Externally no marked change takes place in the development of the nymphs, so far as the genitalia are concerned, except in the ventral sclerites at the tip of the abdomen The seventh abdominal sternite shows the most marked changes in development, and by observing its shape and size the males and females may be distinguished as early as the third instar. In the first and second instars it is practically impossible to distinguish any difference, and it is quite difficult sometimes in the third instar Beginning with the fourth instar, however, it is an easy matter to distinguish the sexes. The other sclerites caudad of the seventh sternite also show considerable development in the fourth and fifth instars. It is in these instars that the eighth abdominal spiracle is especially visible. (See Pl. LVII.) At the tip of the abdomen in all the instars is a rather large, flaplike sclerite ventrad of the anal opening, called by Hoppe, 1912, the analkonus. It must represent the sternite of the eleventh segment. This structure entirely disappears at the end of the fifth instar. (See Pl. LVII, Fig. 3, *A*.)

In the female the seventh sternite is prolonged caudally, while in the male it is more rectangular. The sclerites of the sternal side of the ninth, tenth and eleventh segments, including the developing gonapophyses, also seem to be represented. The styli, *Sty*, are distinctly visible as little darkened projections. The pair just cephalad of these is represented by a mere point, as at *e* in plate LVII, figure 4. The ovipositors seem to be represented by small, rectangular areas, *d*, in figure 4, and the sternites of the eighth, ninth and the tenth segments by *a, c, e,* respectively.

In the male the developing genitalia have no external counterparts in the nymphs, so far as could be seen. Only the sternites have corresponding sclerites represented. (See 9, 10, *A*, in Fig. 3, Pl. LVII.)

The caudal part of the tip of the abdomen in both males and females, on the dorsal surface, shows little if any indication of segmental arrangement, but is covered with patches of setæ as in the

adult. These setæ probably aid the bug in keeping its equilibrium in the water and in giving it more floating ability.

INTERNAL. An attempt to dissect out the developing genitalia and study their forms was made. This was done with the expectation that it might throw some light on the structures as found in the adult. Since the parts were soft, and for the most part not held intact by coverings of chitin, it was a rather difficult undertaking. Some evidence was obtained from the fifth-instar nymphs and a mere trifle from the fourth. In the earlier stages nothing could be distinguished since the parts were not clearly differentiated. The materials were such as had been preserved in 85 per cent alcohol. Some had been fixed in picrosulphuric acid.

For the female the parts as dissected out are represented in figure 6, plate LVII, for the fifth instar. The fourth instar appeared much the same except that it was smaller. The origins of the parts could not be distinguished clearly, but seemed to bear out the studies and conclusions on the adult.

In the study of the fourth instar very little could be distinguished of the developing genitalia of the male. The parts were very much all one mass. With the fifth instar some detail was available. Figure 5, plate LVII, represents what was found. Here it is seen that the œdagus appears bifurcate at the tip, showing its development from a pair of gonapophyses, as has been found in other studies. The pair of gonapophyses not seen in the adult seems to be present here as at *A*, figure 5. It must fuse with the pair forming the œdagus. The pair representing the genital claspers in development is quite evident, as at *c*, figure 5.

The locations of the gonapophyses were the same as were found in the adults. It is desirable that further and quite careful studies be made to verify these results.

COMPARATIVE STUDIES.

OVIPOSITORS. A brief study of the ovipositors, to see if the number and position of the teethlike setæ might be of specific value, has been made and is appended here. To get absolutely reliable data these studies would have to be extended over a much larger number of individuals. Not more than three or four individuals were used from any one of the places named, and in some cases just one, because of the lack of sufficient material.

The number of teeth on the cutting edge of the ovipositor was taken for one count. The number of teeth not on the regular cutting edge of the ovipositor, but situated on the sides and out of alignment,

as at *A* in plate LVI, was taken as another count. The counts were made under transmitted light with oil immersion or high power magnification.

Locality label and specific name.	*Number on edge.*	*Number on side.*	*Number of specimens examined.*
Bryant's Bog Douglas Lake, Michigan (*B. macrotibialis* paratype specimen)	40 and 41	—	1
Douglas county, Kansas (*B. margaritacea*)	33 and 35	2 or 3	4
Chile, South America (Undetermined)	50 and 48	4	1
Bryant's Bog, Douglas lake, Michigan (Undetermined),	40 and 38	4	2
Benson, Minn. (Undetermined)	37	none	3
Stubb's pond, Douglas county, Kansas (Undetermined),	37	2 or 3	3
Superstition mountains, Arizona (Undetermined)	35	2 or 3	1
Sedge Point pool, Douglas lake, Michigan (Undetermined)	30 and 31	2 or 3	2

It will be seen that the number of teeth on the cutting edge of the ovipositor varies from 30 to 50, and that there is some variation in the number not on the cutting edge. There are also other characters of the ovipositors, which may be taken as indicating specific value. (See Pl. LVI.)

GENITAL CLASPERS AND SPINES. A little more detailed and extensive study of the genital claspers and the spine at the caudo-sinistral margin of the seventh tergite was made than of the ovipositors. The results are shown on plates LIII and LIV. The plates are largely self-explanatory. Only a few specimens of each were used but the shapes and sizes seemed to be fairly constant for the specimens from any given locality.

It will be noted that there are some differences, but often they are so slight that they would not be considered of much specific value The pair of claspers of a species of *Buenoa* from Chile, South America, will be seen to be one about which there could be no mistake in saying that they are distinctly specific. Another one from the Superstition mountains, Arizona, is readily distinguished by its size. There are at least three types of spines which are quite distinct. While it may be said that these characters as a group are not of specific value, yet it must be acknowledged that there are several individual instances in which they are, no doubt, of specific value. Such a study as this needs to be worked out with a larger number of cases before absolute conclusions may be drawn.

SOUND DEVICES. The following notes have been made of the number of setaceous teeth and spines on the sound-producing areas of the anterior legs of the males:

B. margaritacea: Right tibia, 27 teeth; left tibia, 26 teeth; one spine on the coxa.

B. elegans: Forty teeth on each tibia; 2 spines on the coxa.

Buenoa from Chile: Right tibia, 8 teeth (probably some broken off in hand-

ling; left tibia, 12 teeth. A row of spines on femur just opposite the teeth on the tibia. No visible sound-device area on the femur, and no spine visible at corresponding place on the coxa, as in the above species.

Other differences have been noted which have specific value. In specimens taken about Lawrence, Kan., and which superficially look much like *B. margaritacea*, the males have two constantly differing forms of the sound-producing areas on the front femora. Specimens from other localities have similar differences. Evidently there are several species not yet fully described.

INTERNAL MORPHOLOGY.

Brief and preliminary studies were made of the internal morphology in connection with working out the tracheal system and the associated hæmoglobin cells. Some notes and plates are given herewith showing the parts and structures which are not greatly different from what is found in other Hemiptera.

DIGESTIVE TRACT.

For a general idea of the digestive tract see plate XLVIII, figure 1. When dissected out it measures about two and one-half times the length of the body. Beginning at the head we find the salivary glands divided into four groups or clusters. The foremost group lies partly in the head next the beak and in the prothorax. The hindmost group lies farther back and mostly in the metathorax. Riley and Johannsen have figured the salivary glands of *Notonecta maculata* after Bugnion and Popoff in their "Handbook of Medical Entomology," page 28. For *Buenoa* practically the same sort of structure has been found.

The œsophagus is a long, slender tube as it extends through the thorax and beneath the dorsal apodeme as figured in plate XLIV. What appears to be the proventriculus is reached in the caudal part of the metathorax and extends for some distance into the abdomen. Sectioning shows no chitinous teeth or anything of the kind in this region, but the walls are very thick and seem to be of a glandular nature. The lumen at this point is little larger than in the œsophagus. No gastric cæca seem to be present, and if such ever have a glandular function, that function must be performed in *Buenoa* by the thickened glandular walls of the proventriculus.

The mid-intestine is considerably coiled about, but not as long as might be expected in an herbivorous animal. Toward the caudal end it appears to be somewhat segmented, and is supplied with a great many trachea.

Evidently the processes of digestion and absorption are quite active at that point.

The hind-intestine is rather small, as might be expected of a predaceous bug living on animal foods, but possesses a fairly large rectal cæcum, as do some herbivorous insects. At its anterior end arise the Malpighian tubules. These are brownish, knotty-appearing tubes, and when traced out thoroughly are seen to form two loops as shown in plate XLVIII. The two ends of each loop appear to arise from the intestine laterally at about the same point. If rectal glands are present they were not observed.

NERVOUS SYSTEM.

The ventral nervous system appears to possess five abdominal ganglia from which the nerves can be seen branching to the various organs. Its location in the abdomen is ventral, and on the mesal line below the hæmoglobin cell clusters, as shown in plates XLIV and XLV. In the thorax it is much larger and thicker, due to the three or four large ganglia there. Some cephalization of the ganglia of the entire ventral cord is observed. In the head the ocular lobes are quite large.

CIRCULATORY SYSTEM.

The dorsal vessel, or heart, is found to be in the position indicated in plates XLIV and XLV. The enlarged portion next the dorsal line of the second to fifth segments of the abdomen has been mentioned on page 294. The aorta is seen to be much depressed in position by the dorsal apodemes of the thorax and to extend forward just dorsad of the œsophagus to the supraœsophageal ganglion, as is usually the case. Several times in dissecting, the heart was observed pulsating in the salt solution in which the specimen was immersed. Also, in the observations described on page 294 it was seen pulsing. The rate of pulse was about 70 per minute. The pericardial cells about the wing muscles seemed to have no definite arrangement in the specimens examined.

Locy, 1884, observed pulsating organs in the proximal ends of the tibia of a number of water bugs, including *Notonecta,* and described and figured those in *Benacus, Nepa,* and one or two others. He concluded that the organs which he observed were entirely separate from the musculature of the legs, and observed under magnification the corpuscles of the insect's blood flowing through them. He stated that the pulsating organs which he observed aided the flow of the blood in both directions through the extremities. The same phe-

nomena were observed in this laboratory in the prothoracic and mesothoracic legs of *Buenoa*. This might be expected, since it is a genus related to *Notonecta*.

GENITAL SYSTEM.

The internal organs of the genital systems of both male and female are figured in plate XLVIII. In the male the testes appear to consist of three parts, and are located ventrally just caudad in the abdomen of the second abdominal spiracle. (See, also, Pls. XLIV and XLV.) Large tracheæ from that spiracle supply them and seem to support them. If a ligament of the testis or ovary was present in the specimens dissected it must have been very inconspicuous. Each part of the testis appears to consist of a long tube coiled up in a roll and inclosed in membranous covering. The vas deferens is a long tube of uniform diameter about two and one-half inches in length. The bug's body is only about 7 mm. long. This tube is coiled with its mate from the opposite side into a great mass in the caudal part of the abdomen. (See Pl. XLVIII.) Just before the two vasa deferentia extend caudad of this mass they unite into the ejaculatory duct. Near this union are three short-knobbed, glandlike organs, probably accessory glands.

In the female the ovaries are located and supported by the tracheal tubes from the second abdominal spiracle, as was found for the testes in the male. The oviducts leading caudad from them, dorsad and between the hæmoglobin cell groups, unite directly into the bursa copulatrix. From the dorsal surface of this, and at an enlarged portion, extends a long, coiled tube inclosed in a delicate membrane and with a knoblike enlargment at the end. This tube is about four times the length of the body of the adult insect, and appears to be the spermatheca. (See Pl. XLVIII, Fig. 2, *Sp.*)

RESPIRATORY SYSTEM.

The respiratory system is not greatly different in most respects from what is found in most Hemiptera. It consists in the main of ten pairs of spiracles in the nymphs, one of which is atrophied in the adults; longitudinal tracheæ on each side, from the caudal atrophied spiracle of the eighth abdominal segment to the mesothoracic spiracle of the suture between the mesothorax and the prothorax; and of transverse tracheæ between the corresponding spiracles of each segment, except possibly the metathorax, and the first and second abdominal segments. The longitudinal connection

between the first and second abdominal spiracles is quite difficult to trace. The branching and rebranching and the extraordinary development of the tracheæ in the thorax is remarkable.

L'ORGANE TRACHEO-PARENCHYMATEUX. Ferriere, 1914, described and figured what he called, "L'Organe tracheo-parenchymateux de quelques Hemipteres aquatiques." These were two white organs on either side of the dorsal vessel in the thorax which were penetrated with a great number of small tracheæ. He found them in *Nepa, Ranatra,* and *Naucoris;* but did not find them either in *Corixa* or *Notonecta.* He seems to have concluded that they were degenerate groups of muscles in the thorax of insects which no longer used their wings as they once did in their phylogenetic development.

In plate XLIV is figured a remarkable group of tracheæ which in the thorax seems to be homologous with "L'Organe tracheo-parenchymateux" of Ferriere. There are other such groups of tracheæ in the thorax of *Buenoa,* but none so large and with such remarkable development of tracheæ as this one. The plate shows just one of them in lateral view. There are two just like the one figured. Each one lies in an almost vertical plane extending dorso-ventrally on either side of a mesal rectangular group of muscles which extend caudo-cephalad between the anterior margins of the mesothorax and the large dorsal apodeme at the anterior margin of the metathorax. By carefully dissecting a dried fresh specimen of *Buenoa* they are most easily seen. Each one of these groups of tracheæ seems to supply the muscles between them. This must have been once true of the organs figured by Ferriere. The organs he described were white. These also are whitish when the tracheæ are filled with air. The organs he described in *Ranatra* were examined in specimens of *Ranatra fusca* and found to bear a marked resemblance to those of *Buenoa.*

HÆMOGLOBIN CELLS.

REFERENCES. The hæmoglobin cells, as mentioned in the introduction, are closely associated with the tracheal system and should be considered in connection with it. Definite hæmoglobin cells, or cells containing hæmoglobin, were first found in the larvæ of *Gastrophilus equi.* It appears they have been found in no other insect except *Buenoa.** They were first observed and described from the larvæ of the horse botfly by Enderlein (25), 1899. The next year, 1900, they were more fully described and figured by A.

* Poisson: Pages 192-201, Archives de Zoölogie Experimentale, T. 65, Fas. 4, 1926, describes these cells in the genus *Anisops.*

Prenant (64), of Nancy, France. Other short articles were written about these cells just previous to that time and immediately after. In 1909 Portier, in "Physologie de L'appareil respiratoire des larves D'Oestre," gave about the last original investigation concerning these cells that is found in the literature. Berlese, 1909, in "Gli Insetti" refers to the literature and sums up the knowledge then acquired. Rollet as early as 1861 had discovered that there was hæmoglobin in the common blood worm or Chironomus larva. But in the Chironomus larva there seem to be no cells in which the hæmoglobin is confined. Dr. H. B. Hungerford reported hæmoglobin in the cells of *Buenoa* in 1922.

ARRANGEMENT. The red cells in the abdomen of *Buenoa*, which in living material is a bright scarlet color and dark red or black in museum specimens, occupies in some cross sections of the body nearly one-third of the abdominal space. The groups into which these cells are clustered surround and are attached to the tracheæ which come from the spiracles of the third to the seventh abdominal segments, inclusive. (See Pls. XLIV and XLVI.) In plate XLVI will be seen something of the grouping of the cell clusters as they usually appear in specimens when the dorsum is removed.

There are two rows of the cell groups on each side of the meson. One row is on the outside from the meson. Through this row the main tracheal trunks arise dorsally from the spiracles beneath to the longitudinal tracheæ and digestive tract above. This outer row consists mostly of one cell group to each segment of the body. Sometimes the branch trachea leading to the digestive tract leaves the main stem trachea before it emerges above the cell group. This is true of those in the third and fourth segments especially. The others leave the stem above the cell cluster. (See Pl. XLVI.) Some of the cells are attached at the extremities of branch tracheæ coming from the main trunk arising through their midst. Others are attached at the extremities of tracheæ coming directly from the spiracle. (See XLVII, Fig. 2.)

The inner row of cell groups next the meson consists entirely of cells in groups about the ends of treelike branched tracheæ arising from the same spiracles as do the main trunk tracheæ. They are arranged in one group to each segment except in the fifth and sixth segments. There the spiracles are especially large, and the internal development of the tracheæ and the attached cells is correspondingly large. Often in the fifth and sixth segments in the inner row there may be four or five groups of the cells.

In segment three of the abdomen the cell groups are rather small, due to the fact that this part of the abdomen is largely occupied by the ovaries or testes which arise or are supported by tracheæ coming from the second abdominal spiracle. Also the length of this segment is much shortened, and near the meson the large apodeme with their attached muscles extend caudad from the first sternite.

Again the cell groups are somewhat smaller in the seventh abdominal segment. Here the reason is that the space is mostly taken over by either the large coil of vasa deferentia in the male or the coiled spermatheca in the female. Also, in the female especially, the more caudal segments are here retracted and occupy much of the space.

The tracheæ branching from the atrophied eighth abdominal spiracles do not supply hæmoglobin cells, but extend to the ovarian tubes or vasa deferentia and the parts of the genital segments.

Each cell group so named is loosely held together by a delicate peritoneal membrane. This delicate membrane may be demonstrated in two ways, either by gross dissection or by sectioning the abdomen. It is seldom in gross dissection that it will be seen, except when particular care is taken to shave off the group very carefully in physiological salt solution close to the spiracle. Usually it is broken and does not hold the cells together in a manner to attract attention. Many gross dissections were made before it was discovered.

The group with its surrounding membrane was transferred with a pipette and suspended in salt solution to a slide and studied under low-power microscope for some time before it would break. Frequently renewing the evaporating salt solution, by adding some that was fresh with a pipette, prolonged the time the membrane would hold intact. In such a position it appeared holding the cells in a cluster more or less spherical, as shown in plate XLVII, figure 3. After a time some of the cells would begin to hæmolyze, probably due to too much salt in the solution, which was constantly evaporating. The hæmolyzed material, like little reddish droplets of oil, would bubble out from the cluster through openings in the surrounding membrane. It was stated in the preceding paragraph that the cells seemed losely held together by the surrounding membrane. It may have been that there were natural openings in the membrane at frequent intervals, allowing the body fluid or blood to freely circulate between the hæmoglobin containing cells. Such

is a matter which needs more demonstration. The membrane itself may have broken down in the supersaturated salt solution or it may have been slightly punctured in removing. This technique was repated several times, with always the same result.

On most every slide containing a number of sections of that part of the abdomen containing the hæmoglobin cells there are some which plainly show the inclosing membrane. Most of the slides in this study have been stained with eosin and Delafield's hæmatoxylin stains. But few nuclei have been observed in the membrane.

STRUCTURE. Plate XLVII, figure 1, shows the structure of the cell and the manner of its attachment to the trachea. The large tracheæ branch and rebranch into smaller tracheæ until the whole mass has much the appearance in form of a tree. At the end of the twigs or tracheoles (small tracheæ without tænidia, perhaps), the cells containing the hæmoglobin are attached. Each individual cell has about the shape shown in the figure, an oblong cell tapering at each end.

The masses of cells taken from fresh bugs were mounted in newly-made physiological salt solution on a slide and picked apart under a binocular with fine needles, so that the individual cells could be distinctly seen. A cover slip was then dropped over them, and they were carefully and repeatedly studied with the oil immersion objective and No. 10 oculars on a Bausch-Lomb compound binocular microscope. In this manner many details could be observed in the evidently yet-living cells. Most of the details were the same as were afterwards observed in sectioned material.

A number of tracheoles branch out from the point of attachment of the cell to the small trachea and pass out in almost parallel but curving lines toward the opposite pointed end of the cell. Cross sectioning shows most of these tracheoles to be just underneath the periphery of the cell. The periphery of the cell is a rather tough membranous covering retaining the protoplasm within. In the living, unstained material the nucleus is distinctly seen in the cytoplasm as a globular, lighter-colored area. Within it can be seen some darker areas but not in definite outline. The rest of the cell shows, aside from the nucleus and the tracheoles, an even, slightly pinkish color, due to the oxyhæmoglobin present.

In the fixed, mounted and stained material the same details of structure can be seen as given above. But the nucleus stains darkly with Delafield's hæmatoxylin, and the remainder of the cytoplasm an even pinkish color with eosin stain. In addition small granular-like specks are seen distributed throughout the cytoplasm and some

larger, dark-stained bodies. Some may be fat bodies and some the by-products of the cell metabolism. (See plate XLVII.)

In most if not every detail of structure the cells are very much like those figured by Prenant in his "Notes Cytologiques" on the hæmoglobin cells of the larvæ of *Gastrophilus equi.*

DEVELOPMENT. Of the development of the hæmoglobin cells in *Gastrophilus equi,* Prenant, 1900, thought there were certain fat cells about the tracheæ which became modified into hæmoglobin cells. He gave a figure in one of the plates he produced, "planche XV, figure 3" of his "Notes Cytólogiques VI," showing the gradations and all the stages between the fat cell proper and the cell containing hæmoglobin. But Prenant seems to have thought they were ectodermal in origin. Adipose tissue is usually considered mesodermal in origin. Berlese, 1909, in summing up the matter in "Gli Insetti," discusses the probability of whether they were ectodermal or mesodermal in origin.

Portier, 1909, in writing of the physiology of the respiratory apparatus of the horse botfly larva, said: "On a distingue deux sortes de cellules trachaeles; les unes anterieures, dont le protoplasma est bourre de reserves graisseuses; les autres posterieures, teintes d'hemoglobine." He did not discuss the origin and development of these cells, but simply made the above statement, and then discussed certain experiments which he tried on the living larvæ with various colored oils and other substances.

It has been noted that there are also two kinds of cells about the tracheæ in the abdomen of *Buenoa,* the fat cells and the hæmoglobin cells. This suggests that in this case, also, the hæmoglobin cells may be fat cells modified as in the horse botfly larvæ. The fat cells in the adult are found more especially around the tracheæ leading from the second abdominal spiracle. Also, some are found around the tracheæ leading from the other abdominal spiracles. Around these other spiracles they occur as a rather thin coating of fat just beneath the red cell clusters. Since the fat cells and the hæmoglobin cells are in close proximity, it may be that there is a transitional stage between them like that found by Prenant in the case of *Gastrophilus equi.* If such is true, it must occur in the early nymphal stages, for it has not been seen to happen in either the adults, the late instars, or in the developing embryo.

In the article announcing the discovery of the hæmoglobin cells it was stated that they "are found in the abdomen of the embryo not yet hatched." These studies have not verified that statement con-

clusively. Because eggs and first- and second-instar nymphs could not be secured till too late for a full investigation of the fresh material, this part of the work has not been completed satisfactorily.

Studies were made of the developing embryo within the egg. Eggs gathered May 14, and others laid in the laboratory May 15 and on the days succeeding this, were the ones most carefully studied. They were dissected from the plant by splitting the stem and pulling the halves away from the sides of the eggs. Then they were kept in small petrie dishes in pond water together with a little Ceratophyllum. This condition did not usually interfere with the development of the eggs if they were kept in water all the time and not subjected too long to heat and strong light. Eggs exposed to the strong light and heat of the binocular-stage light for more than two or three minutes never hatched. Embryos well developed within the egg chorion were observed when so placed to revolve round and round on their horizontal axis by movements with the tip of the abdomen. This must have been for the purpose of not leaving the one side exposed to the strong light and heat too long. They could not be seen to so revolve at other times. The studies were made with reflected and transmitted light under the binocular, and also under a Bausch-Lomb compound binocular microscope equipped with 16-mm. objective No. 10 oculars, and Abbe condenser. With a pipette the eggs were placed for examination in a drop of water on a slide.

For the first two days after the eggs were laid little differentiation could be seen from any position. Then certain of the large yolk bodies, scattered promiscuously about through the egg, became a light pink in color, and the germ band could be seen along the ventral side. The day following this, or the second day, the germ band was further developed and the appendages became very evident. The yolk bodies were of a slightly brighter red, and began to arrange themselves in rows dorso-ventrally along the sides of the egg. The development continued during the following days; but the red bodies, or yolk granules as they appeared to be, lost their coloring, except a few near the caudal end of the egg and a few near the center of the abdomen. The pink coloring matter became more and more diffused throughout the entire egg, except in those parts on the ventral side where the germ band and its appendages were most developed. Next the red coloring began to appear as two dorso-ventral red marks on the sides of the cephalic end of the egg. The red yolk bodies entirely disappeared, and the diffused pinkish color throughout the egg gradually disappeared as the eyespots took on more and more color,

until about three days before hatching. At this stage no more pinkish color remained diffused through the egg. The yolk bodies on the dorsal side of the abdomen were nearly all gone, and two black streaks along the venter, representing the hairs to cover the ventral air channel, had developed. The eyes were bright red and well developed. But no indication of hæmoglobin cells could be seen in the abdomen. From this time until hatching little change was seen, except the disappearance of the yolk bodies in the abdomen. Most of the eggs hatched May 24 to 26.

In the first instar nymphs no indication of the hæmoglobin cells was seen. Many of them had a greenish tint in the fore part of the abdomen, but not the slightest trace of any red cells. The eyes were rather bright red when the nymph first hatched, but turned to darker shades of red as the nymph grew older. In the older nymphs and adults the eyes are brownish or black.

Egg fixed in Perenyi's fluid, chromonitric acid, and preserved in 85 per cent alcohol during the various stages of development, often show the eyespots and other red markings within the chorion still in their natural or nearly natural color. The color varies from a bright to a rather dark red.

Nymphal instars and adults, fixed in any of the various fixatives and preserved in 85 per cent alcohol, show the hæmoglobin cells to have turned from the bright-red color of the living material to a dark brown or black. Since the red coloring matter in the eggs does not change color when fixed and preserved, it must be an entirely different substance from that in the hæmoglobin cells. It seems very probable then, that the "red cells . . . found in the abdomen of the embryo not yet hatched," were not hæmoglobin cells, but the yolk or similar bodies containing the coloring substance which later accumulated in the eyes of the developing embryo. The study is being continued, and further observations may cause different conclusions.

Little more can be said at this stage of the investigation concerning the development of the hæmoglobin cells, except that preserved, sectioned, and living materials have shown them to be well developed in the third instar and those following.

PHYSIOLOGICAL FUNCTION. Examination of the literature concerning the hæmoglobin or tracheal cells of *Gastrophilus equi* has revealed very little about their physiological function. Doctor Hungerford wrote concerning those of *Buenoa:* "These large clumps of red cells are most intimately connected with the respiratory system, and might well serve to fix and store the oxygen as a reserve supply."

The fact that hæmoglobin is found present in the cells may seem *prima facie* evidence that their function is for the purpose given in the above quotation. To the writer's knowledge hæmoglobin serves no other purpose than to loosely unite with oxygen when brought in contact with it, and then release it again when surrounding conditions demand such. In the vertebrates it unites with oxygen in the lungs, is carried to other parts of the body, and there, as the conditions demand, is released. This cannot be true in *Buenoa,* for the hæmoglobin is confined to certain attached cells which do not circulate in a medium like the blood of the vertebrates.

There may be two or three possible ways in which the hæmoglobin of the cells in *Buenoa* function for the insect's body. In each case most of the oxygen must get to the hæmoglobin from the air through the tracheæ and their extensions which lead to and penetrate the cells. If this is not true, then it is a question why there should be such an elaborate system leading into them. Some oxygen may penetrate through the tissues, for it has been shown that gases, including oxygen, penetrate directly through chitin and other tissues. (Moore, 1916.) Some small insects have little or no tracheal system, although they are enveloped in a chitinous covering. At least we know there is a direct connection between the outside air and the interior of the hæmoglobin cells.

The first of the possible ways for the hæmoglobin in the cells to function is that the oxygen may reach them through the tracheæ and the tracheoles, be fixed there temporarily, and then be distributed to the tissues by the blood which bathes the hæmoglobin cells inside the membrane which surrounds them. It was indicated on page 306, in the discussion of the arrangement of the hæmoglobin cells, that the blood might so bathe them, but was not conclusively shown.

The second way may be similar to the first, except that the oxygen is diffused through the surrounding membrane to the blood and then distributed to the tissues. Since the membrane is very thin, the oxygen would very likely penetrate it more easily than it has been shown to do to chitin.

The first or second possible processes may be used wholly or in part. Muttkowski (53), 1921, by a series of experiments, demonstrated a respiratory protein in a great number of insects, but did not use *Buenoa.* He did use *Notonecta,* a related genus. The respira-

tory protein he called hemocyanin, and its nucleus he found to be copper. All the insects he used showed the presence of hemocyanin. It is probably also present in *Buenoa*. So a respiratory function for the blood of *Buenoa* must be included as being quite probable.

The third possible way is the same as the first and second, except that the oxygen may be released back to the air from which it first came, and from that through the tracheæ to the tissues. This is considered much more likely, but the first or second possible ways may be used slightly in coördination with it. This third way is supported by observations on the habits of the insect.

Muttkowski (52), 1920, points out that the air in the tracheæ of Odonata naiads is the same as in the water, approximately 65 parts nitrogen to 35 parts oxygen, as compared to 80 parts nitrogen and 20 parts oxygen in the atmosphere. When *Buenoa* has been beneath the surface of the water for some time the proportions of nitrogen and oxygen may be nearly the same as in the Odonata or other insects with a closed tracheal system. Now it will be recalled that in the discussion of the swimming habits of *Buenoa*, given on page 277 of this paper, that the insect uses effort at first after coming to the surface in order to keep itself below, and then later has to use effort to keep from sinking. The likely reason for this is that the oxygen of the fresh air secured at the surface unites with the hæmoglobin in the red cells and reduces the volume of the air buoying up the insect, so that it is soon well poised in the water. Then as the oxygen of the air in the tracheæ and ventral air chamber is used by the tissues, some of the oxygen from the hæmoglobin cells may be released back to the air in the ventral air chamber sufficient, perhaps, to keep the proportions of near 65 parts nitrogen and 35 parts oxygen as found in the surrounding water. Some of it may pass also to the respiratory protein of the blood, as was indicated by Muttkowski. After the oxygen supply has become depleted then the insect must return to the surface. The great length of time which the insect swims in almost perfect poise, compared to the lengths of time in which it is using effort either to get below the surface or to keep from sinking to the bottom, indicates very much that the above-described process is what occurs.

Krogh has devised an apparatus by which he was able to measure extremely minute quantities of gases. Tests can probably be made to give further evidence with respect to the air at different times in the ventral air chamber of *Buenoa*.

EXPERIMENTS.

A great number of observations were made and several experiments regarding the respiratory function of *Buenoa*. The following are typical of the swimming habits:

EXPERIMENT No. 1.

After a cool night, May 14, 1924, with the temperature in the laboratory at the time of the observations around 58 degrees Fahrenheit, notes were made on three adults of the species *B. margaritacea*. Each was in a separate jar of water sitting on a well-lighted window sill. The observations started at 9:30 a. m.

Bug No. 1:

At 9:35 a. m. it came with effort to the surface for air. Before obtaining the air it was heavier than the water, for it sank when it ceased momentarily to swim. After it obtained air at the surface it had to use considerable effort for nearly two minutes in order to regain its customary poise in the water. It maintained its poise with little effort until about 9:52, and then began to use more and more effort to keep from sinking to the bottom.

At 9:54:10 a. m. it touched the surface for renewed air supply, and then repeated the behavior as before.

Bug No. 2:

At 9:38:05 a. m. it touched the surface for air and then acted as did bug No. 1.

At 9:57 a. m. it again touched surface for air.

Bug No. 3:

At 9:44:35 a. m. it touched surface for air and repeated behavior of bugs Nos. 1 and 2.

At 10:04:45 a. m. it touched surface for air again, etc.

At 10:12 a. m. the observations ceased. None of the bugs remained at the surface for air more than four seconds. The periods between the times when they touched the surface film for air were as follows: Bug No. 1, 19 minutes 10 seconds; bug No. 2, 18 minutes 55 seconds; bug No. 3, 20 minutes 10 seconds.

The periods during which they were striving to keep below the surface after taking air could not be measured exactly, but were about 3 minutes for each bug.

The periods during which they were noticeably striving to keep from sinking to the bottom were about 1 minute each.

The periods during which they were swimming well poised in the water, due to the functioning of the hæmoglobin cells, were approximately as follows: Bug No. 1, 15 minutes; bug No. 2, 13 minutes; bug No. 3, 15 minutes.

No other creature in the insect world, so far as is known, can swim so nicely poised for anything like such a length of time. Surely this ability of *B. margaritacea* and the other *Buenoa* has a meaning all its own. The periods of time as given in the preceding experiment vary somewhat with temperature and other conditions. The shortest time observed for *Buenoa* to remain below without coming to the surface for air was 1 minute 10 seconds; and the longest time was 31 minutes 43 seconds with bugs collected from under ice and brought to the laboratory.

EXPERIMENT No. 2.

SUBJECT: *Buenoa margaritacea* Bueno.

OBJECT: Behavior when kept from the surface air.

PROCESS: Bugs were collected and put in aquarium jars at ordinary room temperatures. A wire screen made to fit closely to the bottom of the aquarium jar was dropped over the bug in the jar with as little disturbance to the bug as possible. The bug could not then return to the surface for air. The bugs were kept in laboratory two weeks or more previous to the experiment. Tap water was used and the bugs placed in the experimental jar at 7:30 of the preceding evening. The experiment began at 8:17 a. m., November 20, 1923. Different bugs were used for each trial.

Results: In the first trial it was found that the period of time, beginning when the bug last obtained air at the surface until it ceased activity, was 4 minutes and 10 seconds. Data given as follows:

Time.	*Intervals and notes.*
8:17:10 p. m.	Left surface film was covered with wire screen immediately.
	Interval: 50 seconds.
8:18 p. m.	Tried frantically to get to air.
	Interval: 3 minutes 20 seconds.
8:21:20 p. m.	No further activity except a faint kick now and then.
	Interval: 40 seconds.
8:22 p. m.	Screen lifted and insect brought to surface and left floating.
	Interval: 3 minutes.
8:25 p. m.	Recovered activity again.

Results: In the second trial, time till activity ceased was 8 minutes 45 seconds.

Time.	*Intervals and notes.*
8:42 p. m.	Bug at surface film, went below, covered.
	Interval: 2 minutes 10 seconds.
8:44:10 p. m.	Tried for air.
	Interval: 3 minutes 30 seconds.
8:45:30 p. m.	Frantic.
	Interval: 3 minutes 30 seconds.
8:48 p. m.	Less active.
	Interval: 2 minutes 45 seconds.
8:50:45 p. m.	All activity ceased.
	Interval: 1 minute 45 seconds.
8:52:30 p. m.	Screen removed, bug floated to surface on its side.
	Interval: 45 seconds.
8:53:15 p. m.	Spasms.
	Interval: 1 minute 45 seconds.
8:55 p. m.	Active again.

Results: In the third trial, November 21, 1923, bugs kept in trial jar overnight, undisturbed. Time till activity ceased was 16 minutes 10 seconds.

Time.	*Intervals and notes.*
9:27:35 a. m.	At the surface for air.
	Interval: 9 minutes 10 seconds.
9:36:45 a. m.	Uneasy.
	Interval: 7 minutes.
9:43:45 a. m.	Sank to bottom and became quiescent; kicked some.
	Interval: 10 minutes 45 seconds.
9:54:30 a. m.	Brought to surface.
	Interval: 3 minutes 45 seconds.
9:57:15 a. m.	Active again.

Results: In the fourth trial, time till activity ceased was eighteen minutes, 20 seconds.

Time.	*Intervals and notes.*
10:04:10 a. m.	At surface; put screen over it.
	Interval: 5 minutes 20 seconds.
10:09:30 a. m.	Uneasy.
	Interval: 13 minutes.
10:22:30 a. m.	Sank, and time was not free to finish this trial.

It will be seen that the average interval until activity ceased was 12 minutes 16¼ seconds. The longest interval until activity ceased was eighteen minutes and twenty seconds; and the shortest was 4 minutes 10 seconds. The above experiment may be of some value or significance.

November 11, 1923, a large corixid, *Arctocorixa,* species unknown, was treated in the same manner as the above *Buenoa* were handled. The results are given below and are interesting for comparison: Time till activity ceased 38 minutes 10 seconds.

Time.	*Intervals and notes.*
9:33:45 a. m.	At surface for air; submerged; screen lowered over it without disturbance.
9:37:40 a. m.	Fastened to screen and quiet; wiped wings.
9:43 a. m.	Tried to come up; went down and kept quiet.
9:49 a. m.	Came to top of screen, and attached to a rock.
10:01:55 a. m.	Floated to top and all activity ceased.
10:05:10 a. m.	Brought to surface, where it floated.
10:05:30 a. m.	Revived and began to swim about.

It will be seen that the time until activities ceased with the corixid given above was much longer than with the Buenoa. This fact seems to be in accordance with the observations of others on corixids and notonectids.

EXPERIMENT NO. 3.

A number of observations were made to determine the comparison of the intervals at which various insects of the water come to the surface for air normally. In these experiments the insects were disturbed as little as possible. The first of these was *B. margaritacea* Bueno.

B. margaritacea Bueno: On November 21, 1923, out of 28 observations it was found that the insect came to the surface for air an average of once in 2 minutes 51¼ seconds. The shortest interval was 50 seconds, and the longest interval was 4 minutes 35 seconds. All the observations in this case were made with the same bug as it swam in an aquarium jar at ordinary room temperature.

On November 22, 1923, the same observations were made with another bug. Out of five observations it was found that the average interval was 1 minute 54 seconds. The shortest was 1 minute 40 seconds, and the longest 2 minutes 40 seconds.

On November 27, 1923, the experiment was again repeated with another bug which had been in the aquarium jar for several days. Out of eleven intervals observed, the average was found to be 6 minutes 28 seconds. The shortest was 3 minutes 40 seconds, and the longest 8 minutes 57 seconds.

Of all the observations on *B. margaritacea* Bueno, some 44 altogether, the average was three minutes 48 seconds. The shortest interval was 2 minutes 17 seconds, and the longest was 5 minutes 11 seconds. These results are about

what may be expected on the average in the laboratory. Conditions being different in the ponds, variations would occur there.

Notonecta undulata: The average interval during which this insect stayed under water in the laboratory as observed was 7 minutes 25 seconds. The shortest was 5 minutes 45 seconds, and the longest was 10 minutes 5 seconds.

Arctocorixa sp.: In a few short observations it was found the average interval of immersion without coming to the surface for air was 40 seconds; the longest 49 seconds, and the shortest 8 seconds.

Small dytiscid, species unknown: A few observations were made with this insect as with the preceding, and it was found the average was 3 minutes 56 seconds; the shortest 50 seconds, and the longest 5 minutes 5 seconds.

Cybister fimbriolatus Say: Average 6 minutes 35 seconds; shortest 20 seconds, and longest 11 minutes.

An average table of the intervals at which the preceding insects came to the surface for air may be of interest, and is given here:

Species.	*Average interval: min. sec.*	*Shortest: min. sec.*	*Longest: min. sec.*
B. margaritacea Bueno	3:48	2:17	5:11
Notonecta undulata Say	7:25	5:45	10: 5
Arctocorixa sp.	0:40	0: 8	0:49
Unknown dytiscid, small species	3:56	0:50	5: 5
Cybister fimbriolatus Say	6:35	0:20	11: 0

Ranatra fusca was kept in the laboratory during an entire winter, and frequently observed as to the frequency with which it came to the surface for air, but its habits are unusual in this respect. It hangs for hours sometimes at the surface and at other times lies motionless for just as long at the bottom. No exact time records were made of it.

The above experiments were repeated on a small scale with *Buenoa margaritacea*, a small *Corixa* species, *Arctocorixa* species, *Cybister fimbriolatus*, and a small unknown species of hydrophilid. All were observed under identical conditions of water, temperature, light, oxygen content, and surrounding conditions. The results were practically the same as in the experiments given on the preceding pages.

It will be seen that the above experiments have amounted to very little so far as proving anything about the hæmoglobin cells of *Buenoa* is concerned. It was thought at first there might be a comparison that would be much in favor of *Buenoa* being able to stay beneath the surface of the water for longer periods of time because of the hæmoglobin cells which it possesses. The table above shows that several other insects average longer periods beneath the surface than does *Buenoa*. There is this fact to be taken into consideration, however. *Buenoa* is in somewhat constant motion at all times. Seldom is it seen at rest on the bottom or elsewhere. All the other

water insects, while not hanging at the surface film or objects beneath the surface, are engaged in considerable activity, thus using their oxygen supply quickly. When not active they may cling to objects below the surface and remain there for hours, or periods of time often longer than is the case with *Buenoa*. It is felt, therefore, that a comparison of the intervals of time spent beneath the surface of the water by various insects is of no value so far as proving anything definite about the function of the hæmoglobin cells.

GENERAL SUMMARY.

The results of these studies may be summarized as follows:

1. Some additional life history and biological notes have been added.
2. The external morphology of *Buenoa* has been more fully investigated.
3. Specific characters have been more closely examined and have shown the possibilities of certain heretofore undiscovered structures for specific determinations.
4. The internal morphology has been worked out in a general way.
5. The hæmoglobin cells in *Buenoa* have been further investigated as to structure, development, and function.
6. Certain details of technique in handling such a problem have been further developed.

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EXPLANATION OF PLATES.

PLATE XLIV.

Female. Sinistral view, body section.

External: A, antenna. C, coxa. E, eye. F, femur. I-XI, abdominal segments. 1-9, sternites. Lr, labrum. md, mandibles. Mst, mesothorax. Mt, metathorax. Ovip, ovipositor. Pt, prothorax. S_{1-7}, abdominal spiracles. Sms, mesothoracic spiracle. Smt, metathoracic spiracle, St, sternal gonapophyses. Sty, styli. tc, tarsal claws. Ti, tibia. Tr, trochanter. tr, tarsal segments.

Internal: Ao, aorta. Dv, dorsal vessel or heart. H, hæmoglobin cell groups. IC, internal apodeme, anterior margin of metathorax. Ic, internal apodeme, posterior margin of metathorax. l, longitudinal trachea. M, muscles. Mp. Malpighian tubules. N, ventral nerve cords. Oe, œsophagus. Ovd, oviduct. Ov, ovaries. Pv, proventriculus. Rc, rectal cæcum. Sp, spermatheca. TP, tracheoparenchemateux. tv, transverse trachea.

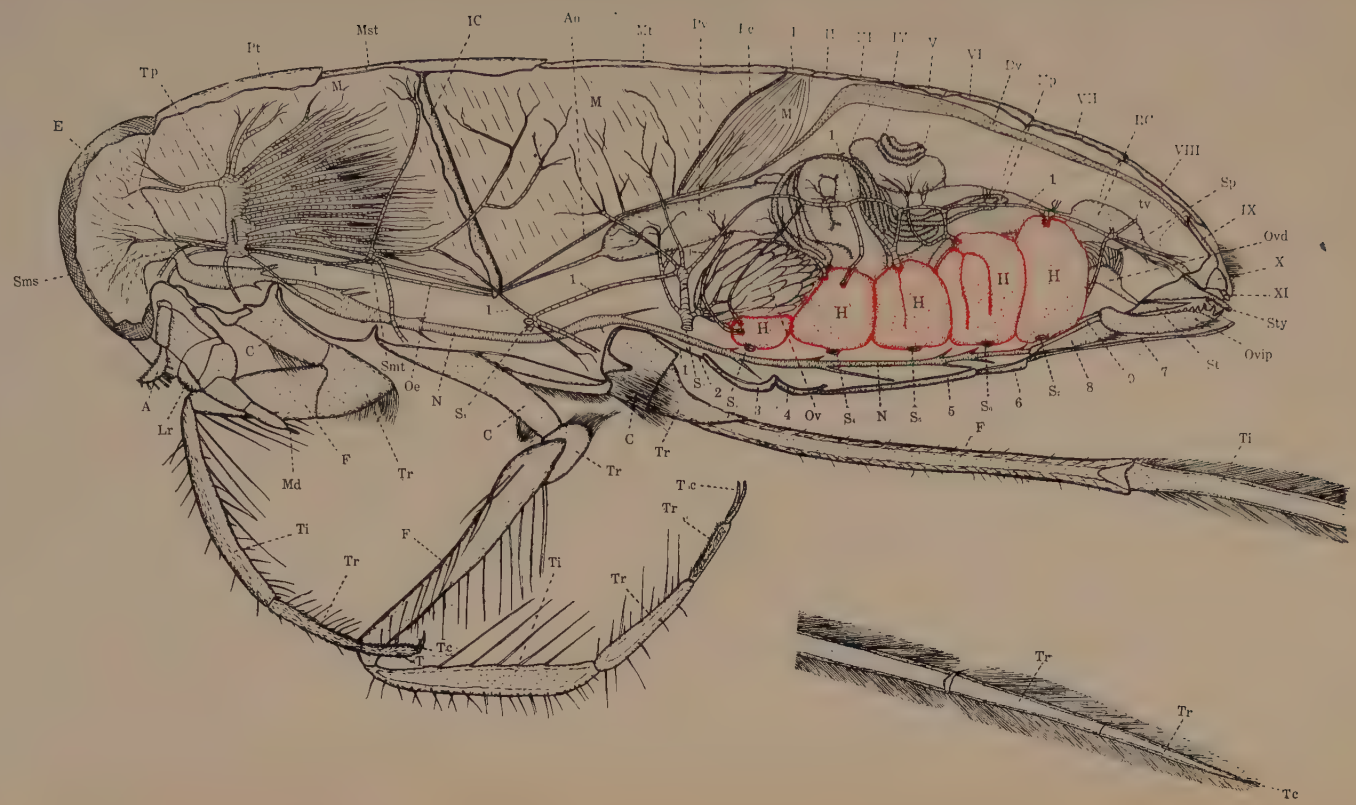
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PLATE XLV.

Male and female. Body sections.

FIG. 1. Head and foreleg of male: A, antenna. Lr, labrum. Sd, sound devices on beak, femur and tibia. Ti, tibia. Tr, trochanter. Tc, tarsal claws.

FIG. 2. Male: Ts, testis. S, spiracle. H, hæmoglobin cell groups.

FIG. 3. Female: H, hæmoglobin cell groups. 1-7, 8, 9, sternites. M, muscles. tv, transverse trachea. Lt, longitudinal trachea. I-XI, abdominal segments. Epi, epimeron. Eps, episternum, S_3-S_6, abdominal spiracles. T, tergum. St, sternal gonapophyses. Ovip, ovipositor. Sty, styli. Ic, internal chitinous process or apodeme, posterior margin of metathorax.

FIG. 4. Metathoracic leg: Tr, trochanter. Tc, tarsal claws.

FIG. 5. Male: Rc, rectal cæcum. Oed, œdagus. Gc, genital claspers. Vd, vas deferens. Ag, accessory glands (?). VIII-XI, abdominal tergites.

PLATE XLV.

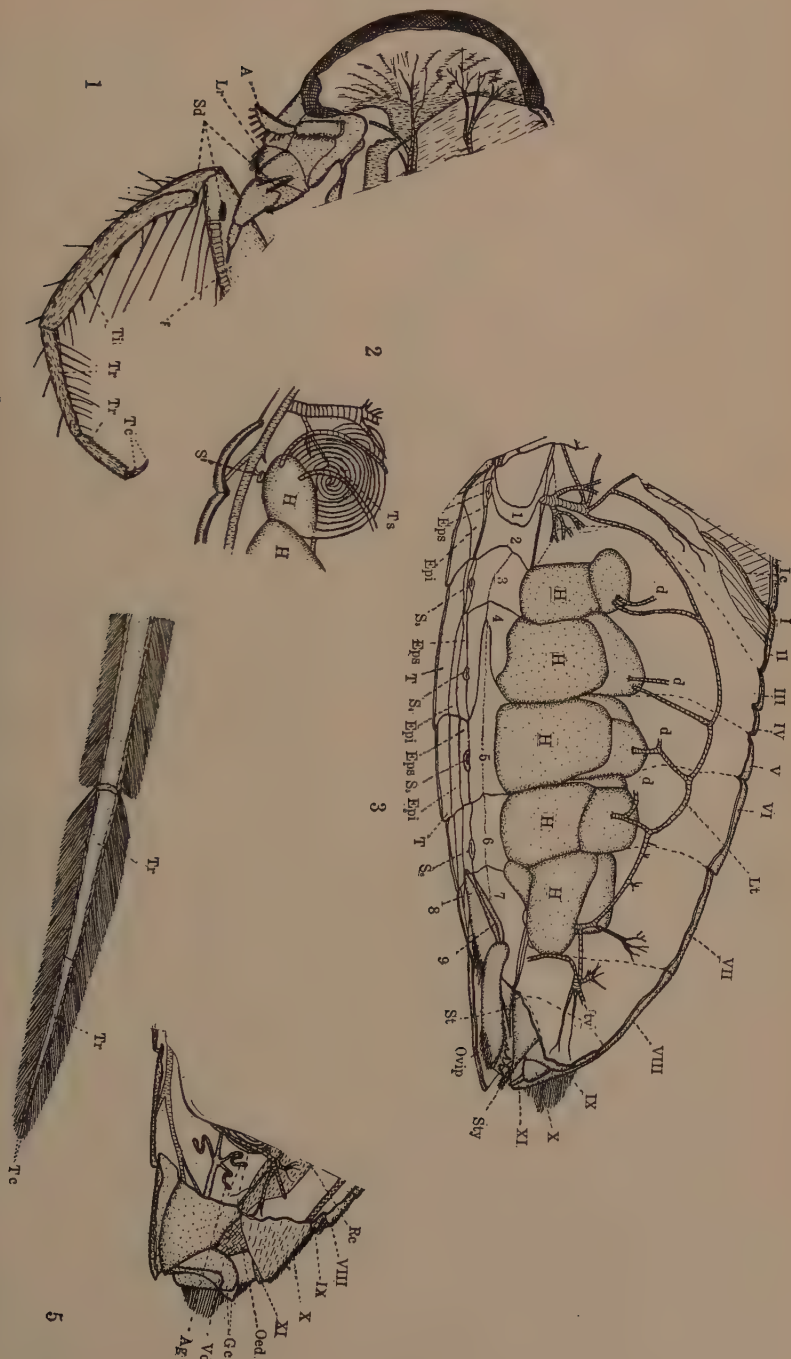
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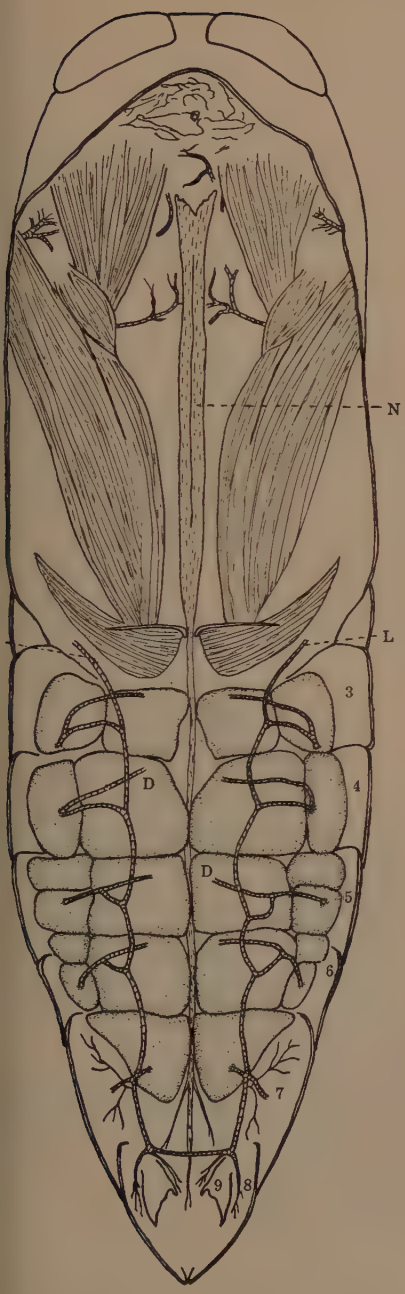
PLATE XLVI.

FIG. 1. Dorsal view of body section, showing arrangement and location of hæmoglobin cell groups in abdomen: D, tracheal branches leading to alimentary tract. L, longitudinal tracheæ. N, ventral nerve system. 3-9, abdominal segments.

FIG. 2. Dorsal view body section, showing ventral sclerites and spiracles from which arise the tracheæ and hæmoglobin cell clusters in the abdomen: Epi, pleurite next tergum. Eps, pleurite next sternum. Ovip, ovipositor. S, spiracles. T, tergum. III-VII, abdominal sternites. 8, 9, terga of seventh and eighth abdominal segments.

PLATE XLVI.



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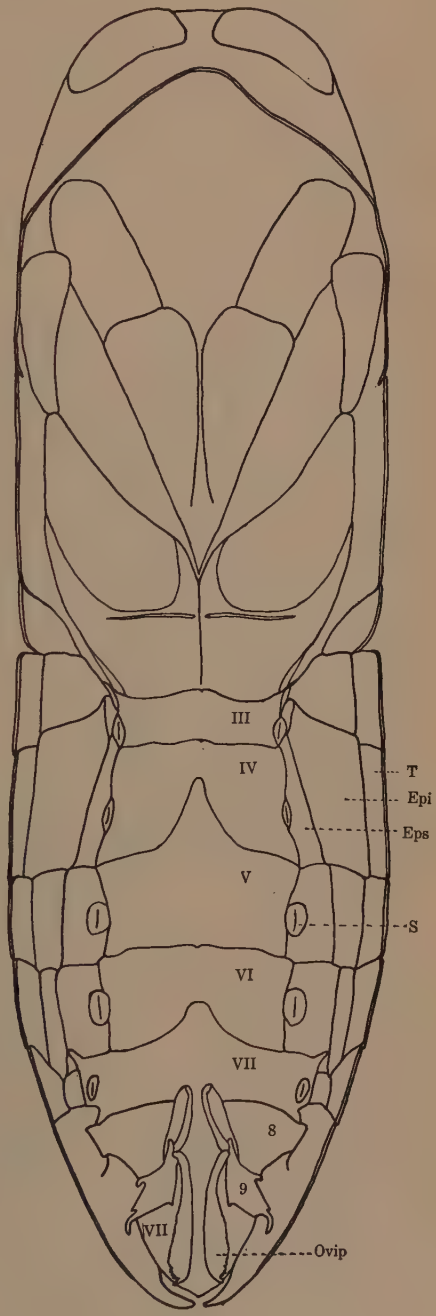

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PLATE XLVII.

FIG. 1. (Oil immersion; in salt solution.) Piece of trachea showing hæmoglobin cell attached: n, nucleus, tr, trachea, with characteristics tænidia, tr. tracheoles. trl′ tracheoles, or small tracheæ with no tænidia.

FIG. 2. (Low power; in salt solution.) Tracheæ from fourth abdominal spiracle, caudal view, sinistral side of body: b, base of tracheæ opening over spiracle below. d, branch leading to digestive tract. Ex, direction of exterior of body. In, direction of interior of body. mt, main trunk of tracheæ, leading to longitudinal tracheæ above. s, stubs of tracheal branches leading into hæmoglobin cell clusters. tv, transverse trachea.

FIG. 3. (Low power; salt solution.) Two clusters of hæmoglobin cells shaved off sharply next the spiracle, showing how clusters are held together by a delicate membrane, as at a and b: a, membrane inclosing large cell cluster. b, membrane inclosing small cell cluster. s, opening of tracheæ to spiracle. tr, tracheæ.

FIG. 4. (Oil immersion; salt solution.) Base of tracheæ about spiracle. Spiracle with peritremes represented by dotted lines.

FIG. 5. (Low power; salt solution projection.) Hæmoglobin cell clusters to show tracheæ and manner of hæmolyzing: a, oillike red globular drops as hæmolyzed from cell cluster. Tr, tracheæ.

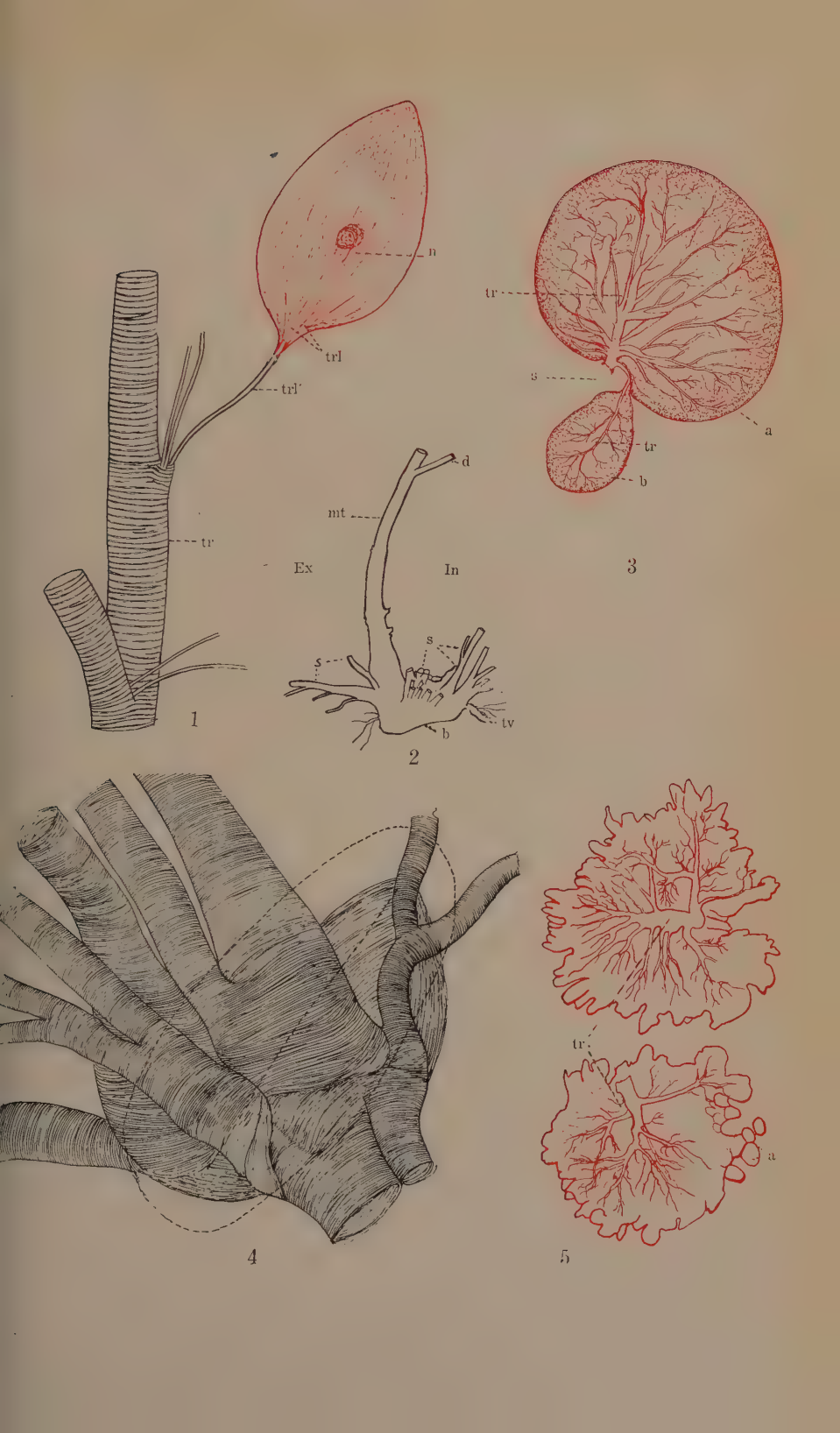
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PLATE XLVIII.

FIG. 1. Digestive tract: Mi, mid intestine. Mp, Malphighian tubules. Oe, œsophagus. Pv, proventriculus. R, rectum. Rc, rectal cæcum. Sal, salivary glands. tr, tracheæ.

FIG. 2. Female genital organs: Bs, bursa copulatrix. Ov, ovaries. Ovid, oviducts. Sp, spermatheca. tr, tracheæ.

FIG. 3. Male genital organs: Ag, accessory glands (?). Ej, ejaculatory duct. Ts, testes. Vd, vas deferens.

PLATE XLVIII.

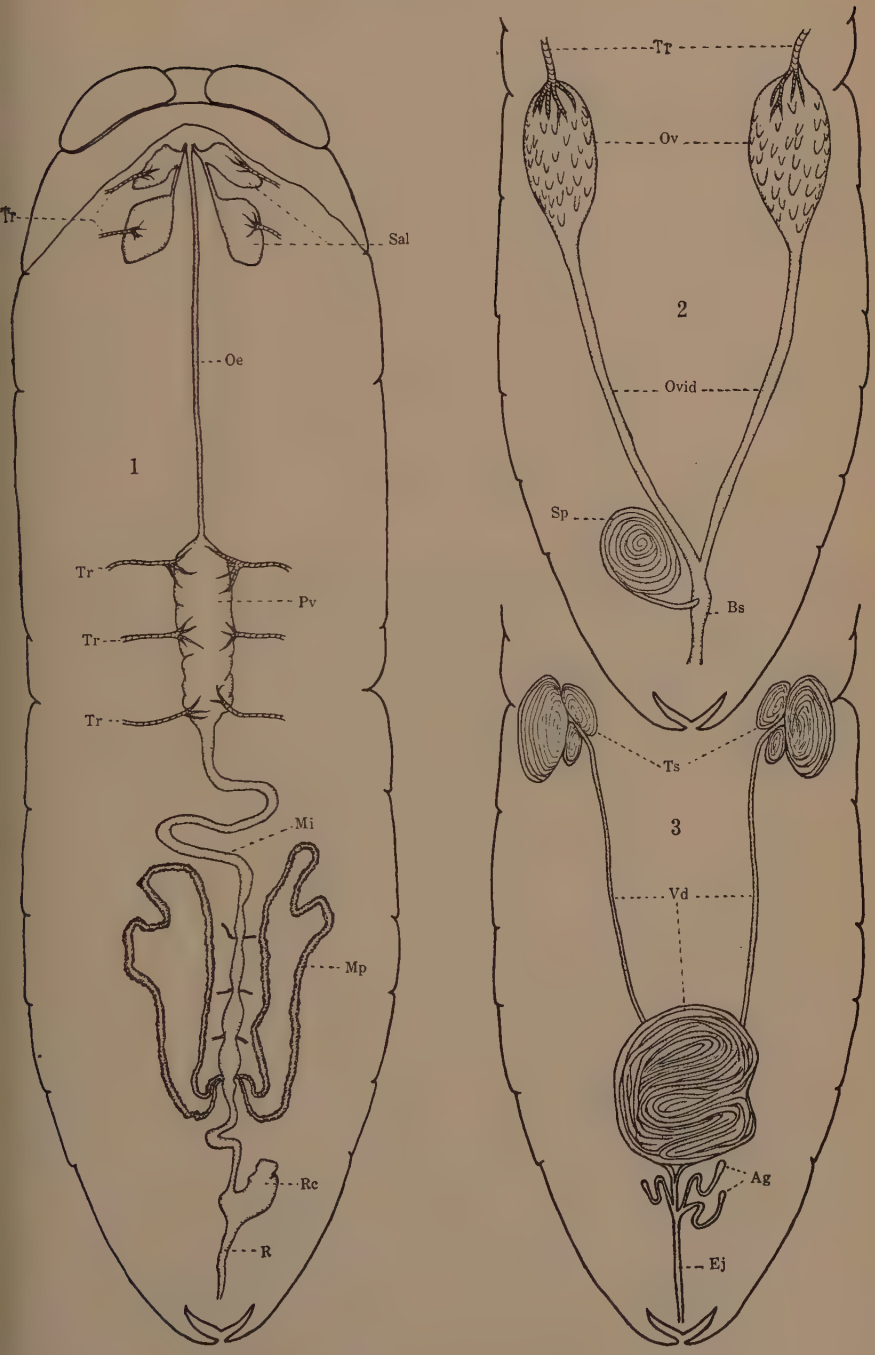


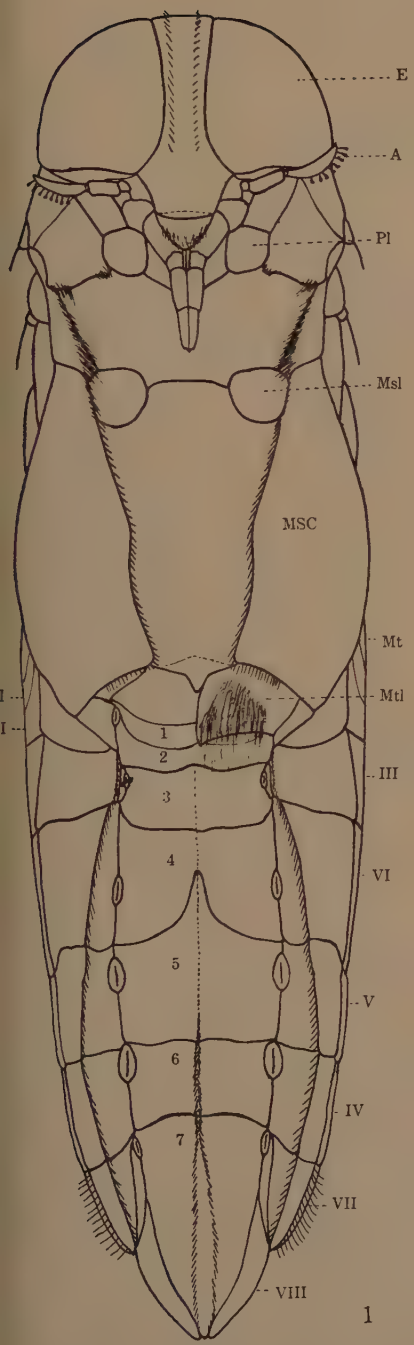
PLATE XLIX.

External anatomy.

FIG. 1. Ventral view female *B. margaritacea:* A, antenna. E, eye. I-VIII, abdominal tergites. 1-7, abdominal sternites. MSC, extension of mesothoracic tergum (Rich). Msl, mesothoracic leg, foramen. Mt, metathorax. Mtl, metathoracic leg, coxa. Pl, prothoracic leg, foramen.

FIG. 2. Dorsal view female *B. margaritacea:* Ao, thinly chitinous area dorsad of dorsal vessel, or heart. I-X, abdominal tergites. Mst, mesothorax. Mt, metathorax. Pt, prothorax. Syn, synthlipsis.

PLATE XLIX.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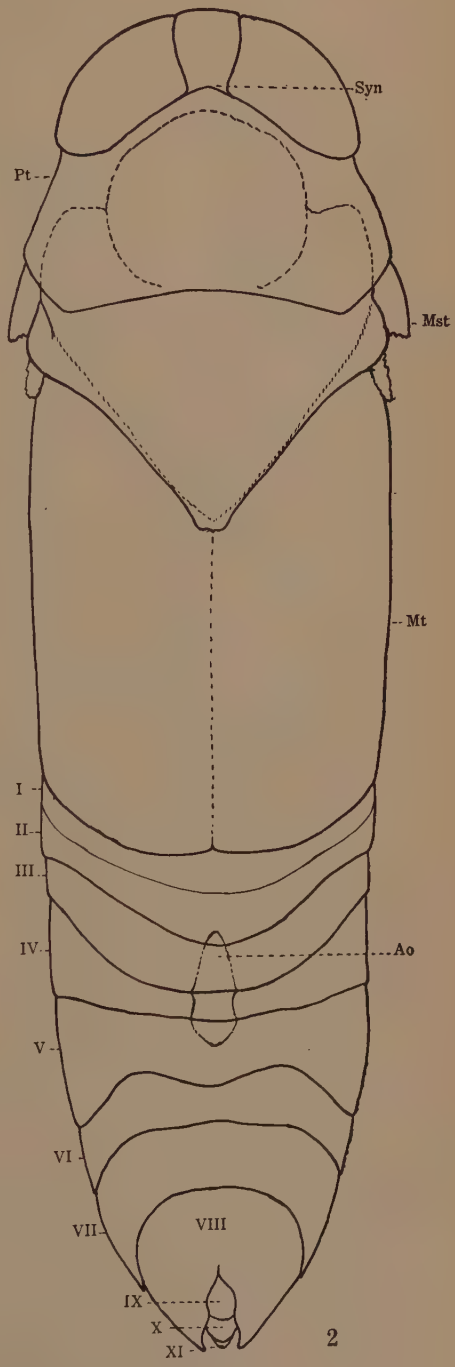
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PLATE L.

External morphology.

FIG. 1. Front wing: B, fastening or hook which hooks into groove on dorsal line at cephalic end of metathorax. L, fastening or hooklike device hooking into similar device at L in Fig. 3. Venation: Costa and subcosta of Comstock-Needham system probably fused along front wing margin. Media and radius fused and extending together into the corium. Cubitus forming back margin of corium. 1st A, 2d A and 3d A combined, found in clavus.

FIG. 2. Hind wing: C, costa. (Comstock-Needham system.) e, probably a remnant of subcosta. F, Subcosta two and radius one. R, Radial sector. M, Media and radius. D, Media and radial sector.

FIG. 3. Side view thorax of male: A, attachment of front wing. A' attachment of hind wing. Ac, antecoxal piece (?). C, coxæ. F, femur. G, glandular opening of the male. L, wing button. MSC, extension of mesothoracic scutellum (Rich). Mst, mesothorax. mst, mesothoracic scutellum. Mt, metathorax. mt, metanotum. Pt, prothorax. pt, pronotum. S, mesothoracic spiracle, beneath. S, first abdominal spiracle. smt, metathoracic spiracle. w, groove into which front wing fits. 1 and 5, mesothoracic pleurites. 2 and 3, metathoracic pleurites. 4, antecoxal piece. 6, mesothoracic sternum.

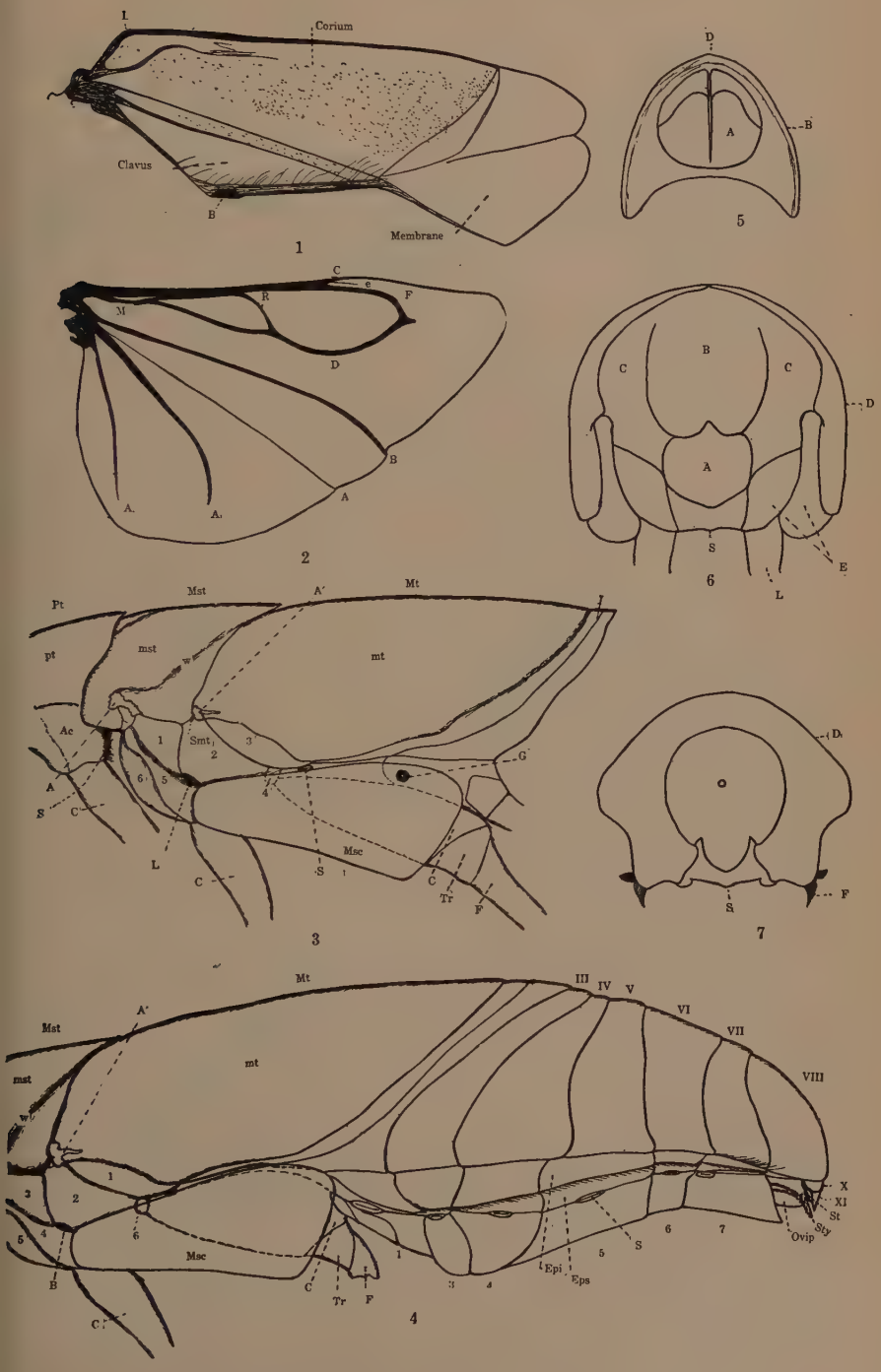
FIG. 4. Sideview thorax and abdomen: A', attachment of metathoracic wing. B, hook for fastening mesothoracic wing. C, coxæ. Epi and Eps, pleurites of abdominal segments. F, femur. I-XI, abdominal segments or tergites MSC, extension of mesothoracic scutellum (Rich). Mst, mesothorax. mst. mesothoracic scutellum. Mt, metathorax. mt, metathoracic tergum. Ovip. ovipositor. S, spiracles. St, gonapophyses. Sty, styli or gonapophyses. Tr trochanter. 1-7 of abdomen, sternites. Of thorax: 1 and 2, pleurites of metathorax. 3 and 4, pleurites of mesothorax. 5, mesothoracic sternum. 6, antecoxal piece of metathorax. w, groove into which margin of front wing fits.

FIG. 5. Caudal view of metathorax: A, apodeme. B, lateral margin of metanotum. D, median dorsal line of metanotum.

FIG. 6. Cephalic end of mesothorax, cephalic view: A, lumen of the mesothorax. B, prescutum. C, scutum. D, scutellum. E, pleurites. L, leg. S sternum.

FIG. 7. Caudal view of prothorax: D, pronotum. F, fringe of setaceous hairs just caudad of mesothoracic spiracle. S, prothoracic sternum.

PLATE L.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g
C
L
C
S
Msc
C
Tr
F
3
D
S
F
7
Mt
III
IV
V
VI
VII
VIII
Mst
A'
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Msc
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Epi
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XI
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Ovip
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4

PLATE LI.

FIG. 1. *B. Margaritacea* (oil immersion). Glandularlike apparatus as pulled out from male showing internal mass of tissues which cling to it: A, mass of tissue mentioned above. B, cells at external opening very darkly chitinized or pigmented. C, opening. L, lines in chitin about opening, apparently sutures.

FIG. 2. *B. margaritacea* (oil immersion). Glandularlike apparatus of male, vertical view from exterior of body.

FIG. 3. (Low power.) Antenna of *B. margaritacea:* A, proximal segment. b, called penultimate segment in literature. c, may represent third segment. d, distal or ultimate segment. e, spatulate, setaceous hairs.

FIG. 4. (8 ocular, 32 objective, Spencer.) Somewhat diagrammatical arrangement of sound apparati of male *B. margaritacea:* A, antenna. C, coxa. Cl, clypeus. E, eye. Ep, epicranium or frons. F, femur. lb, labium. lr, labrum. mx, mandibles and maxillæ. Ti, tibia. Tr, trochanter. Ts, tarsus. Tsc, tarsal claws. 1, thickened angular corner, sides of face. 1′, corresponding setaceous spines and thickened corner of femur. 2, areas on beak prongs. 2′, area on tibia, 3, spur on coxa. 3′, area on femur.

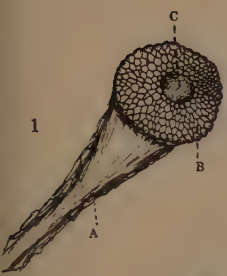
FIG. 5. (Oil immersion, balsam.) Apparatus on front tibia of *B. margaritacea:* A, cephalic end of area. A′, caudal end of area. B, point at which the apparatus is bent in mounting. AA′, whole stridular area. S, row of setaceous spines in view ventrally.

FIG. 6. (Oil immersion, balsam.) Apparatus on mesal face of front femur of male *B. margaritacea:* A—A', stridular area, consisting of transverse thickenings of the chitin. H, strong setaceous spines which aid in forming crib of the front legs for retaining prey. G, glandular-appearing areas in the chitin or just below.

FIG. 7. (Oil immersion, balsam.) Apparatus on prong of beak of *B. margaritacea:* A—A', sound producing area with row of spines or setaceous teeth down the middle of the area.

FIG. 8. (Oil immersion, balsam.) Large setaceous spine on outer face of the front coxa in *B. margaritacea,* for sound production, no doubt: M, proximal margin of coxa next the sternum. S, sound producing spine or spur. s, ordinary setæ on the surface of the coxa around the larger one.

PLATE LI.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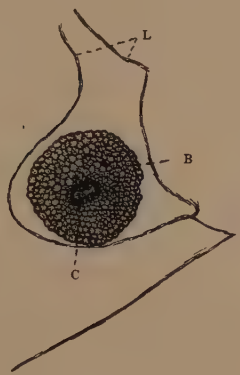
1
B
A


L
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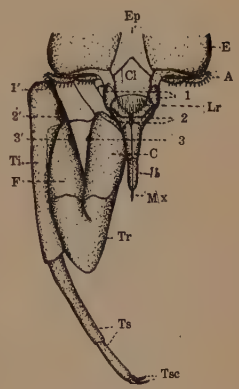
C


Ep
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3'
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Ti
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Mx
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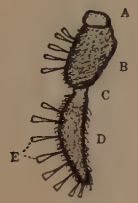
4


A
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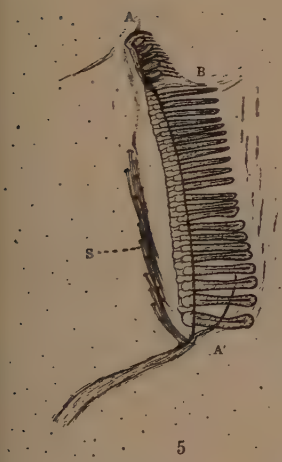
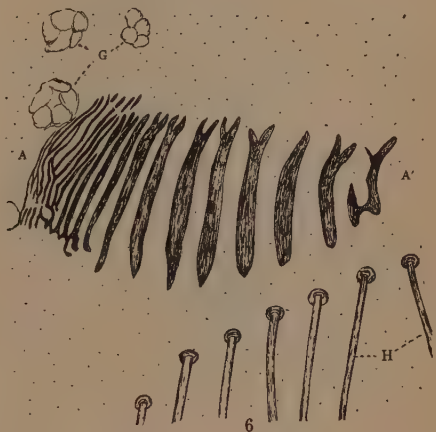
C
D
E
3

2


A
B
S
A'
5


G
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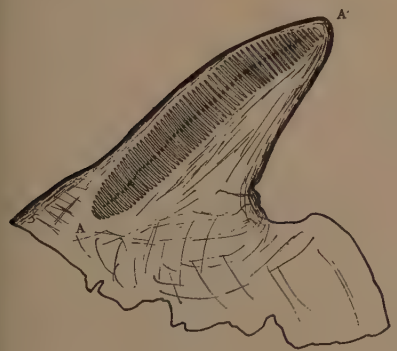
A'
H
6


A'
A
7

8
m
s
S

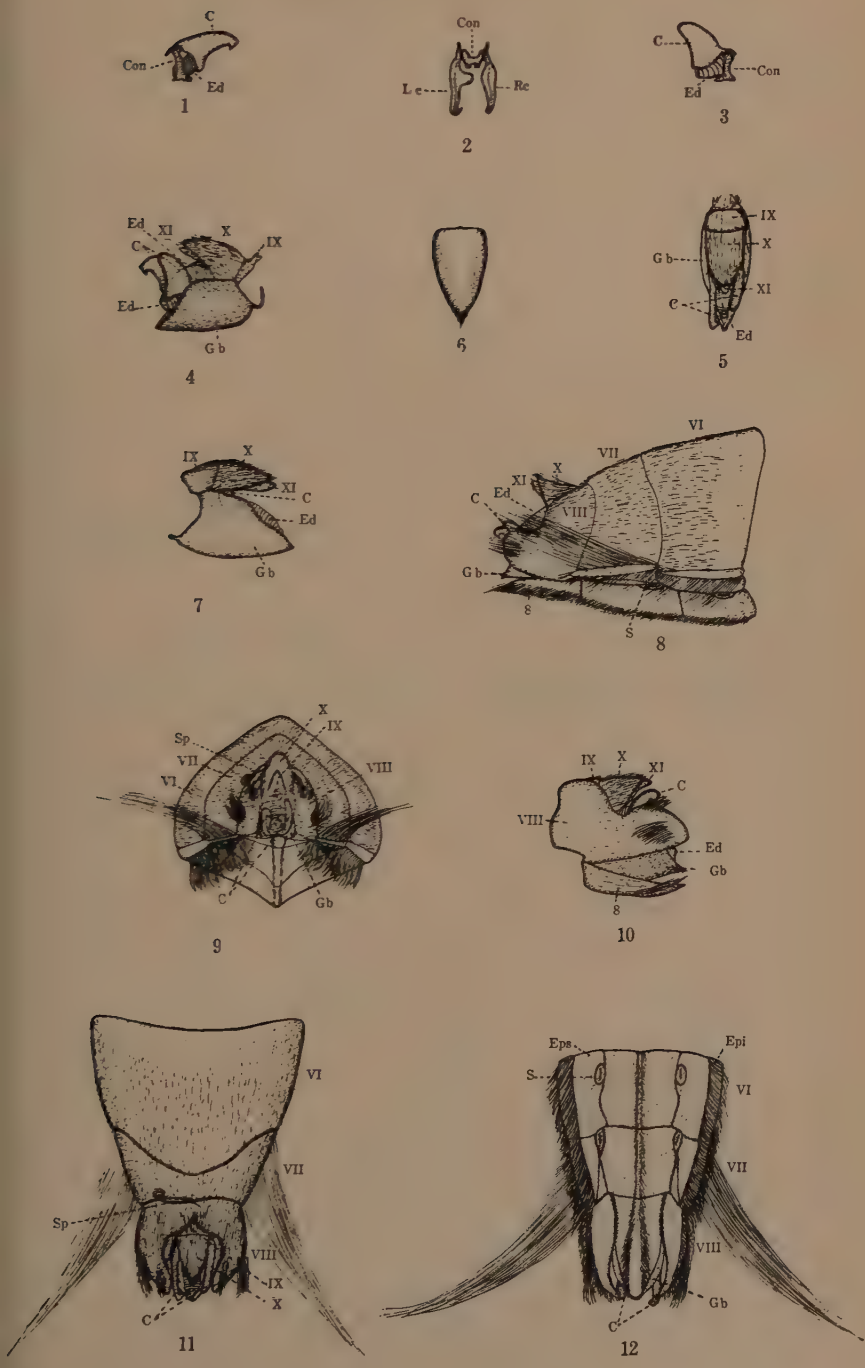
PLATE LII.

Adult male genitalia and abdominal sclerites.

FIG. 1. Left clasper, sinistral view.
FIG. 2. Dorsal view of claspers and connective.
FIG. 3. Right clasper, dextral view.
FIG. 4. Genital bulb, dextral view, claspers extended.
FIG. 5. Genital bulb, dorsal view, ninth abdominal sternite, etc.
FIG. 6. Genital bulb, ventral view, ninth abdominal sternite.
FIG. 7. Genital bulb, sinistral view, claspers retracted.
FIG. 8. Dextro-lateral view, tip of abdomen.
FIG. 9. Caudal view, tip of abdomen.
FIG. 10. Sinistro-lateral view, tip of abdomen.
FIG. 11. Dorsal view, tip of abdomen.
FIG. 12. Ventral view, tip of abdomen.

Lettering: C, clasper, or claspers. Con, connective. Ed, œdagus. Epi, pleurite next tergum. Eps, pleurite next sternum. Gb, genital bulb, ninth abdominal sternite, hypandrium. Lc, left clasper. Rc, right clasper. S, spiracle, Sp, spine on or near caudo-sinistral margin of seventh tergite, of abdomen. VI-XI, abdominal segments, or tergites. 8, eighth abdominal sternite.

PLATE LII.


C
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Re
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Gb
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VII
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Ed
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S
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XI
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Gb
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10
VI
VII
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VIII
IX
X
C
11
Eps
Epi
S
VI
VII
VIII
Gb
C
12

PLATE LIII.

Genital claspers and spines, male.

Left clasper at left of plate, spine of sinistro-caudal margin of seventh tergite at center, and right clasper at right of the plate.

FIG. 1. *Buenoa elegans* Fieb.

FIG. 2. *Buenoa margaritacea* Bueno. Collected at "Union Pacific bridge pond" Douglas county, Kansas, May 15, 1924. Clarence O. Bare.

FIG. 3. *Buenoa macrotibialis* Hungerford. From a paratype specimen loaned by Dr. H. B. Hungerford; collected in Bryant's bog, Douglas lake, Michigan, August 3, 1923.

PLATE LIII.

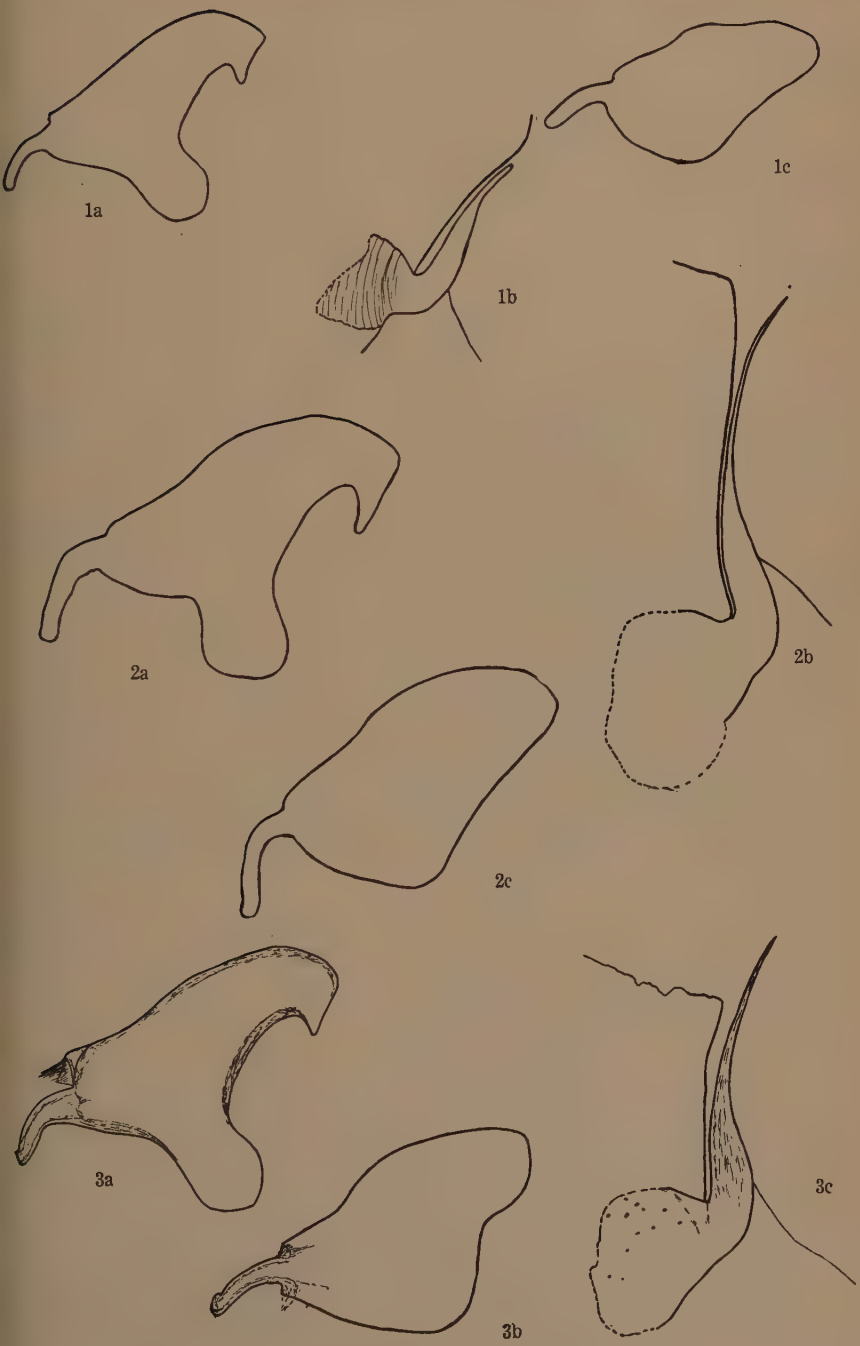

PLATE LIV.

Buenoa males, claspers and spines.

Explanation: Left clasper on left side of plate. Spine from caudo-sinistral margin of seventh abdominal tergite, in middle of the plate. Right clasper on right side of plate, sinistral view. Parts from each individual specimen arranged horizontally across the plate.

Labels borne by the specimens as follows:

No. 1: H. B. Hungerford, Stubb's pond, Douglas county, Kansas, November 27, 1922. *Buenoa scimitra* Bare.

No. 2: H. B. Hungerford, Bryant's bog, Douglas lake, Michigan, July 29, 1923. *Buenoa elegans* Fieb. (?).

No. 3: H. B. Hungerford, Sedge Point pool, Douglas lake, Michigan, July 13, 1923. *Buenoa elegans* Fieb. (?).

No. 4: H. B. Hungerford, Benson, Minn., August 23, 1922. *Buenoa elegans* Fieb. (?).

No. 5: P. A. Glick, Superstition mountains, Arizona, November 7, 1922. *Buenoa arizonis* n. sp. (A full description of this will follow.)

No. 6: Alfredo Faz, Termas Cauquenes, Chile, South America, December 15, 1922. *Buenoa naias* Kirk.

PLATE LIV.

PLATE LV.

Adult female genitalia and abdominal sclerites.

FIG. 1. Sinistral view of genitalia.
FIG. 2. Ventral view of genitalia as shown in Fig. 1.
FIG. 3. Mesal view of ovipositor and adjacent parts.
FIG. 4. Dextral view of tip of abdomen.
FIG. 5. Caudal view of tip of abdomen.
FIG. 6. Sinistral view of tip showing genital parts.
FIG. 7. Caudal view of tip of abdomen.
FIG. 8. Ventral view of tip of abdomen.

Lettering: Epi, pleurite next tergite. Eps, pleurite next sternite. Ovip, ovipositor. S, spiracle. St, gonapophyses (?). Sty, stylets. V-XI, abdominal segments, or tergites. 6-9, abdominal segments, or sternites.

PLATE LV.

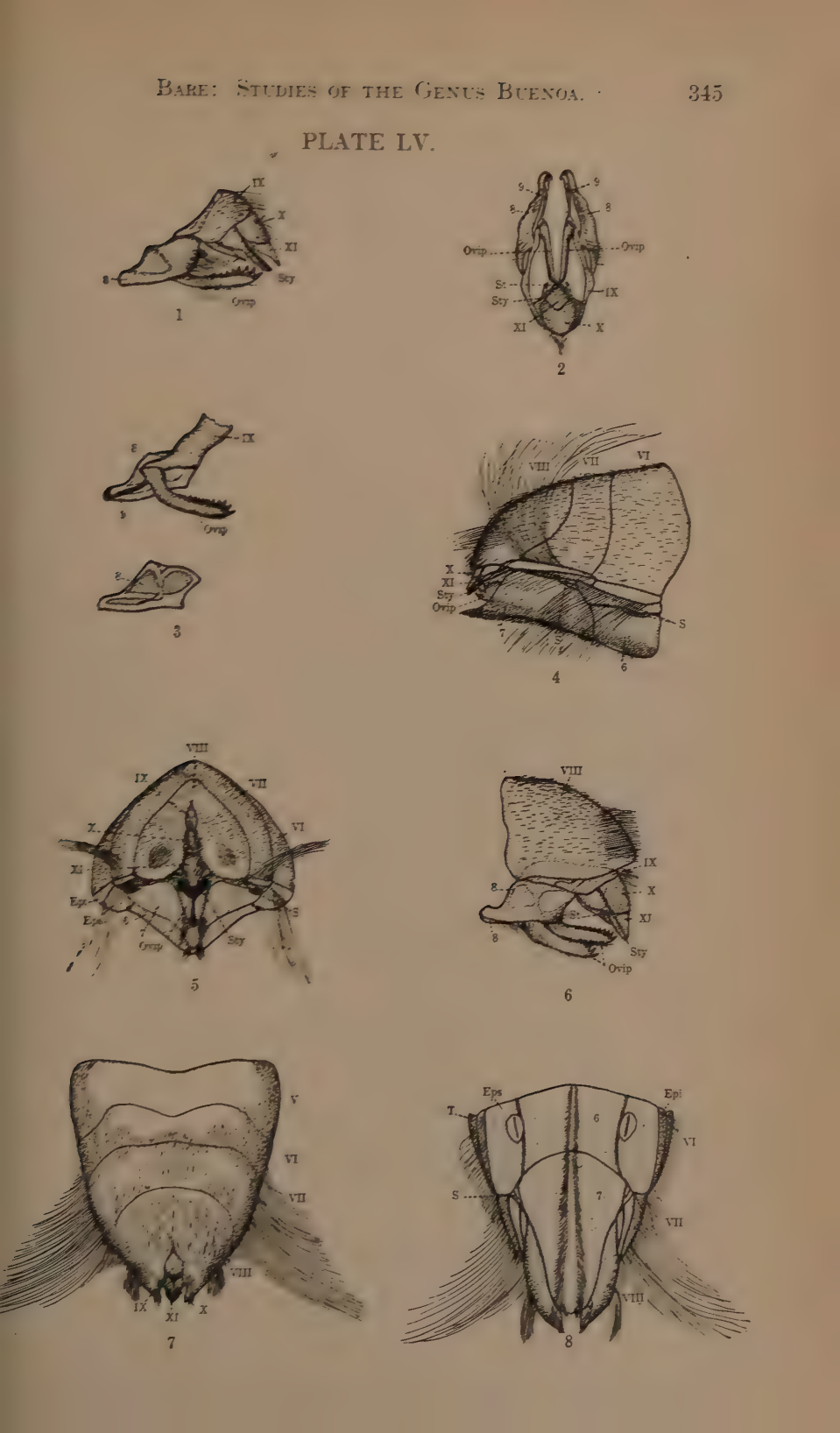
IX
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PLATE LVI.

(Oil immersion, balsam.)

Ovipositor of female *B. margaritacea*, outer lateral view: A, teeth on inner mesal surface. B, setaceous hairs of outer lateral surface. C, toothlike spines, outer lateral surface. D, toothlike spines, inner mesal surface. I-I′, long outer row of setaceous teeth represented by dashes where seen through other chitin. II-II′, shorter inner row of setaceous teeth represented by lighter, thinner lines where seen in section through other chitin. R, row of chitin, may represent ninth abdominal sternite.

PLATE LVI.

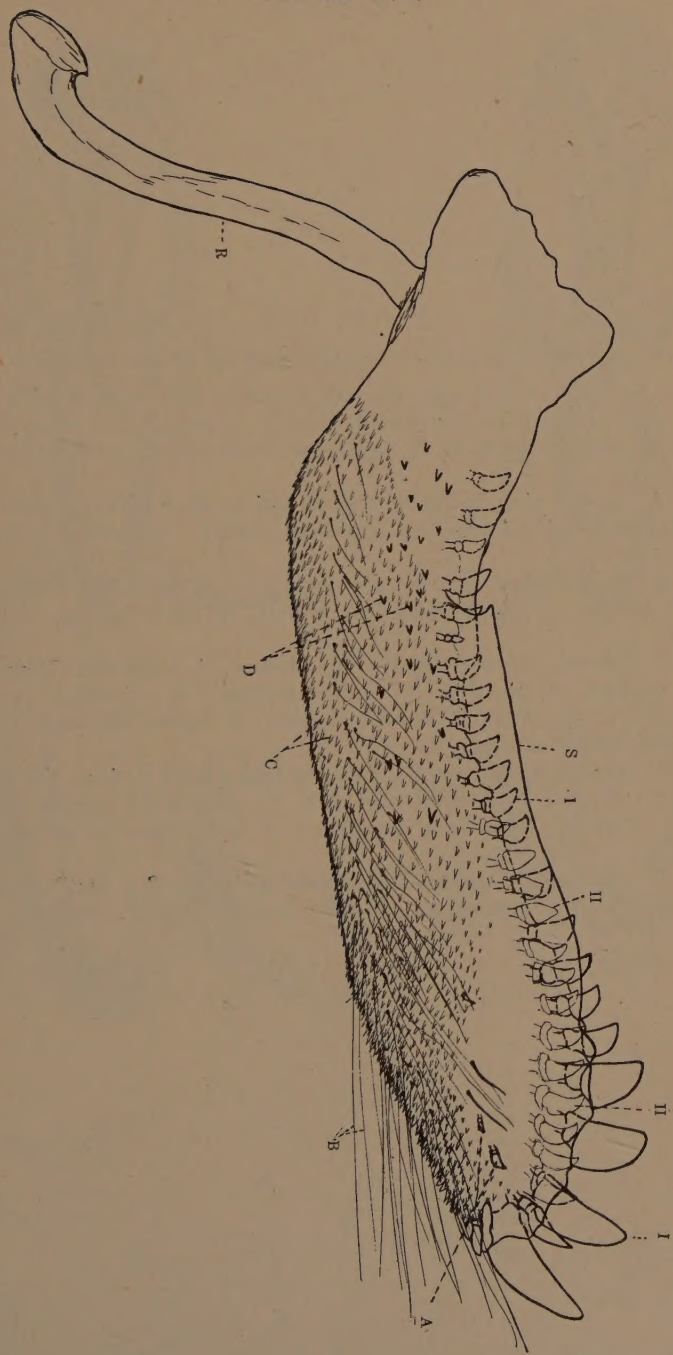


PLATE LVII.

Male and female fifth-instar nymphs, showing genital development.

FIG. 1. Ventral view of male, legs snipped off to allow view of other parts: F, tuft of setaceous hairs, not present in adult. 2, second abdominal spiracle. 7, seventh abdominal sternite. 8, eighth abdominal spiracle, probably nonfunctional.

FIG. 2. Ventral view of female, legs clipped to allow view of other parts: Msc, extension of mesothoracic scutellum (Rich). 7, seventh abdominal sternite.

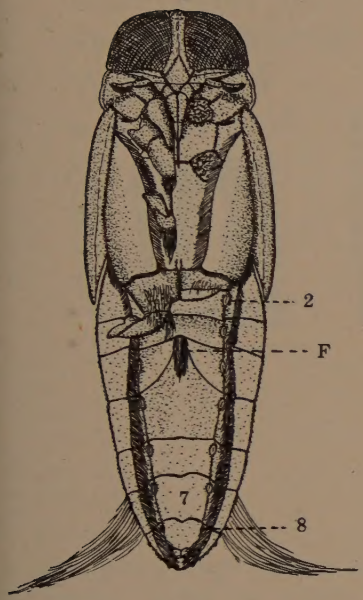
FIG. 3. Ventral view of male tip of abdomen: A, analkonus (Hoppe). 7, seventh sternite. 8, eighth abdominal spiracle. 9, ninth sternite. 10, tenth sternite.

FIG. 4. Ventral view of female tip of abdomen: A, analkonus (Hoppe). a, eighth sternite. b, pleurite. c, ninth ovipositor. d, ovipositor. e, gonapophyses (?). Sty, styli. 7, seventh sternite. 8′, eighth abdominal spiracle. 8t, eighth abdominal tergite.

FIG. 5. Internal development of male genitalia: *Lateral view:* Ed, œdagus. C, clasper. 8 and 9, sternites. *Dorsal view:* C, claspers, Ed, œdagus. 9, ninth sternite. *Tergites*—dorsal view, spread out: 7, 8, 9, tergites of corresponding segments.

FIG. 6. Internal development of female genitalia: Sty, styli. St, gonapophyses (?). Ovip, ovipositors. 8, eighth sternite. IX-XI, tergites.

PLATE LVII.



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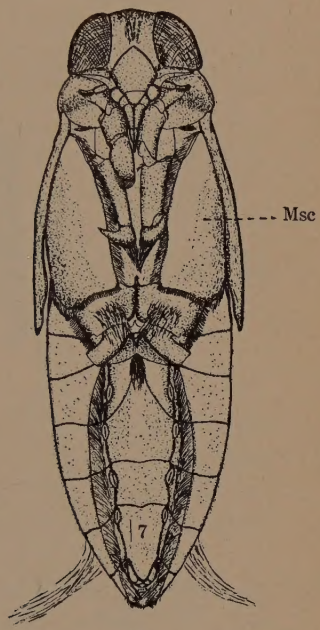

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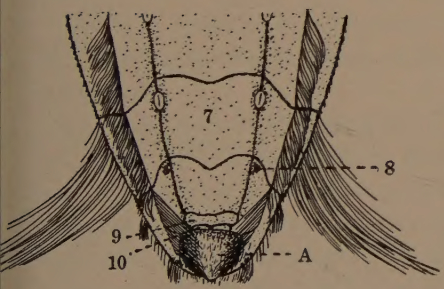

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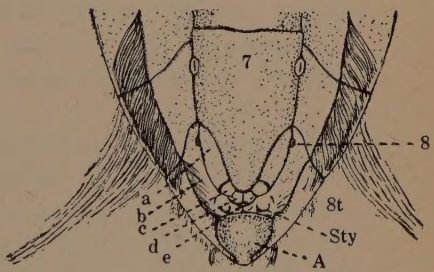

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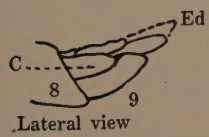
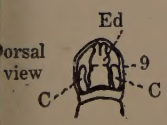
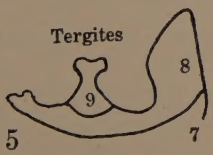


Lateral view





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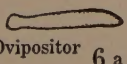
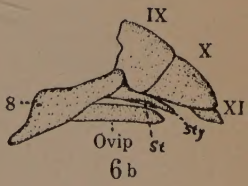


Ovipositor 6 a



6 b